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FOREWORD

The first issue of the Journal of Immune Milk was well received and generated more interest than had been anticipated. Many helpful comments and criticisms were received.

The next issue of the Journal will again emphasize immune milk research from around the world. We will present a series of recent Russian, German, Czecho-Slovakian, and French papers.

Physicians and pharmacists have indicated interest in the legal aspects of immune milk. Therefore, we will also begin publishing information on patents and patent applications dealing with immune milk and the isolated immune milk proteins.

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Director of Research
W. E. Petersen Research Institute
St. Paul, Minnesota
Persistence of Neutralizing Antibody in Milk and Blood of Cows and Goats Following the Instillation of Virus into the Mammary Gland

Charles A. Mitchell, R. V. L. Walker, and G. L. Bannister

Animal Pathology Division, Canada Department of Agriculture, Animal Diseases Research Institute, Hull, P.Q.


Two former papers (3, 4) indicated the results of attempting to propagate Newcastle disease and influenza virus in the bovine mammary gland. It was shown that these two viruses propagated for approximately 2 weeks after which neutralizing antibody could be found in the milk taken from the quarter which was instilled with virus. Later antibody was present in the blood. Antibody both in the mammary gland and in the blood had persisted for approximately 60 days at the time the paper was written. This communication will report on the persistence of neutralizing antibody over a longer period of time.

Persistence of Antibody Formation Resulting From the Propagation of Virus in the Mammary Gland. — Newcastle disease virus into mammary gland of lactating cow. — The "Twiss" strain of Newcastle disease virus was propagated in 9-day-old chick embryos. Immediately after death the fluids were harvested and found to titer $10^3$ (50% embryo death). By means of a teat tube 2 cc of the fluid was instilled through the teat duct into the lactiferous sinus of the right rear quarter of the mammary gland of a lactating cow. The virus propagated in this quarter for approximately 2 weeks and reached a titer in the milk of $10^3$. Virus was not found in milk taken from the other three quarters nor in the blood. Soon thereafter neutralizing antibody was present in the milk of the experimental quarter. A few days later antibody was present in the blood and still later in the milk taken from the other quarters of the gland.

Both the beta inhibition and ordinary neutralization tests were employed for the measurement of antibody. In the former a fixed amount of ten hemagglutinating units of virus was used. Against this was titered dilutions of milk and blood serum. The indicator was the hemagglutination or lack of hemagglutination of chick red cells. The neutralization test employed a fixed amount of milk or blood serum with varying amounts of standardized virus, neutralization being determined by inoculating the mixture into chick embryos which survive if neutralization has taken place.

A summary of the results of these two tests is shown in Table 1.

Influenza A (PR 8) virus into mammary gland of lactating cow. — The virus was propagated in chick embryos and the fluids harvested. The left front quarter of the mammary gland of a lactating cow was instilled with 2 cc of embryo fluids which titered $10^3$. For 2 weeks virus was found in the milk of the instilled quarter but not in the milk of the other quarters nor was it present at any time in the blood. A peak of concentration was reached on the

| Table 1 |
|---|---|
| Sample | Virus | HI test (beta) 2 months | 12 months | 16 months | Serum-virus neutralization test 2 months | 12 months | 16 months |
| Milk, right rear | Newcastle disease | 1:320 | 1:320 | 1:320 | 10-1 | 10-2 | 10-2 |
| Milk, right front (control) | 1:40 | 1:320 | 1:320 | 10-1 | 10-2 | 10-2 |

| Table 2 |
|---|---|
| Sample | Virus | HI test (beta) 2 months | 12 months | 16 months | Serum-virus neutralization test 2 months | 12 months | 16 months |
| Milk, left front | Influenza "A" | 1:1,280 | 1:640 | 1:640 | 10-2 | 10-1 | 10-2 |
| Milk, left rear (control) | 1:160 | 1:320 | 1:320 | 10-2 | 10-1 | 10-2 |
| Blood serum | 1:2,560 | 1:1,280 | 1:640 | 10-2 | 10-1 | 10-2 |
eleventh day and virus in the milk reached $10^{-7}$, a concentration similar to that of the chick embryo fluids employed for instillation.

Antibody was found in the milk of the instilled quarter soon after the disappearance of virus and somewhat later in the blood. The beta and the ordinary neutralization tests were employed. The principal results are summarized in Table 2.

**Influenza A (PR 8) into the mammary gland of a nonlactating cow.** — To ascertain if the activity of the gland had any effect on the modification of virus, a nonlactating cow was used. This animal received in the right front quarter 2 cc of fluids from embryos which had propagated influenza virus A (PR 8). Since milk was not available, daily washings of the quarter were made using 10 cc of sterile physiological saline solution. The right rear quarter was treated similarly. The inoculated quarter yielded virus for the first 8 days which reached a concentration of $10^{-4}$. From the ninth day on, virus could not be demonstrated but neutralizing antibody appeared and reached a high concentration 7 days later. Virus was not obtained from the right rear quarter nor from the blood. Antibody was found in the blood and soon after its discovery in the instilled portion of the gland. The results of later tests are summarized in Table 3.

Because it is more economical to use goats, it was decided to use these animals in a few trials.

**Influenza A (PR 8) virus into mammary gland of lactating goat.** — Influenza A (PR 8) was propagated in chick embryo and fluids harvested. These titered $10^{-6}$. The right half of the goat's gland (unlike the cow the goat has only two parts to the mammary gland) was instilled with 5 cc of these fluids. Milk samples were collected daily from both sections of the gland. Virus propagated in the instilled half and reached a peak of $10^{-6}$ on the fifth day. On the ninth day the agent could no longer be demonstrated. At no time was virus found in the blood.

Four days after the disappearance of virus a low concentration of antibody was demonstrated in the milk. This gradually rose until a peak was reached on the twenty-first day. It continued at this level for approximately 2 months then it dropped slightly. Neutralizing antibody was present in the blood 4 days after its initial appearance in the gland. The titer increased until on the 21st day it reached a concentration similar to that found in the milk from the instilled portion. Milk from the unosed portion of the gland commenced to exhibit antibody only after a high concentration was reached in the blood.

Table 4 summarizes the results of the lactation period of 8 months.

**Newcastle disease virus into mammary gland of lactating goat.** — The "Twiss" strain of Newcastle disease virus was propagated in chick embryos and the fluids harvested. These titered $10^{-7}$ (50% embryo death). Five cc of these fluids was instilled into the right half of the mammary gland. Milk samples taken daily indicated that virus propagated for 9 days, the peak of $10^{-7}$ being reached on the fourth day. Virus was not demonstrated in the milk of the opposite half or in the circulating blood.

Neutralizing antibody was present 5 days after virus ceased to be found in the milk. This increased in concentration and reached a peak on the 21st day. Afterwards it dropped by 50% and remained at this lower level until the lactation period of the animal ceased, approximately 6 months later.

The antibody level of the blood paralleled that of milk. The milk from the opposite half

---

**Table 3.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Virus</th>
<th>HI test (beta)</th>
<th>Serum-virus neutralization test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 month</td>
<td>10 months</td>
</tr>
<tr>
<td>Right front</td>
<td>Influenza &quot;A&quot;</td>
<td>1:280a</td>
<td>1:640</td>
</tr>
<tr>
<td>Right rear</td>
<td>Blood serum</td>
<td>1:40a</td>
<td>1:640</td>
</tr>
</tbody>
</table>

*The HI and SN tests at 1 month were conducted with saline washings of the quarters. As the cow was lactating at the 10th and 16th month after, milk samples were used.*

**Table 4.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Virus</th>
<th>HI test (beta)</th>
<th>Serum-virus neutralization test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (right)</td>
<td>Influenza &quot;A&quot;</td>
<td>1:2,560</td>
<td>1:2,560</td>
</tr>
<tr>
<td>Milk (left)</td>
<td></td>
<td>1:160</td>
<td>1:160</td>
</tr>
<tr>
<td>Blood serum</td>
<td></td>
<td>1:2,560</td>
<td>1:2,560</td>
</tr>
</tbody>
</table>
Table 5.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Virus</th>
<th>HI test (beta)</th>
<th>Serum-virus neutralization test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 month</td>
<td>2 months</td>
</tr>
<tr>
<td>Milk (right)</td>
<td>NCD</td>
<td>1:160</td>
<td>1:160</td>
</tr>
<tr>
<td>Milk (left)</td>
<td></td>
<td>1:20</td>
<td>1:20</td>
</tr>
<tr>
<td>Blood serum</td>
<td></td>
<td>1:160</td>
<td>1:320</td>
</tr>
</tbody>
</table>

showed only a trace of antibody at any time. Tables 5 summarizes results.

Discussion. — The evidence presented indicates that neutralizing antibody may persist in the milk of the gland and in the blood for at least 18 months. That free virus disappears from the milk of the infected gland in 2 weeks or less would appear to indicate that active propagation ceases. If this is true, it is very difficult to account for the stimulus which leads to continuous antibody formation for long periods of time. Previously (4) it was shown that after propagation of virus in the gland, reinfection was impossible. Furthermore, when the virus of poliomyelitis was used (the details of which are not recorded here) propagation did not take place and neutralizing antibody was not found in the milk or blood serum. This seems to indicate that the propagation of virus in the mammary gland provokes the immune mechanism into the production of neutralizing antibody which in itself or together with some other mechanism gives defense against reinfection. What particularly concerns us, here, however, is that the propagation of a virus for a relatively short period of time can stimulate the activity of the immune mechanism for at least 18 months and perhaps for the life of the animal.

In work carried out several years ago (2) on the problem of Brucella infection it was found that removal of the mammary glands of animals that were infected with Brucella abortus and showing a high agglutinating titer was followed by a decline in antibody production. In this case the reason is clear in that the stimulus was removed when the mass of local infection was eradicated.

It seems extraordinary that a stimulus from virus propagated for a relatively short period of time could provoke the immune mechanism for several months unless some factor is involved such as that suggested by Habel (1), the passage of virus from parent to daughter cells during the course of cell division. This led us to design an experiment aimed at the removal of the gland which had once propagated virus, for the purpose of determining if the production of neutralizing antibody ceases. The results will be reported in another paper.

The long term immunity following some virus infections is an interesting feature. Perhaps the phenomenon of producing neutralizing antibody for many months after evidence of the propagation of virus in a local site has ceased may assist in throwing some light on this interesting question.

Literature Cited


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Studies Relating to the Formation of Neutralizing Antibody Following the Propagation of Influenza and Newcastle Disease Virus in the Bovine Mammary Gland

Charles A. Mitchell, R. V. L. Walker, and G. L. Bannister

Contribution from the Animal Pathology Division, Canada Department of Agriculture, Animal Diseases Research Institute, Hull, Quebec.

Manuscript received October 6, 1955.

The assistance of Dr. Eric F. Pallister, who carried out the exacting surgery in this study, is acknowledged with grateful thanks.


Introduction. — In previous communications (1, 2) it was demonstrated that influenza A and Newcastle disease viruses instilled into quarters of the mammary gland of cattle resulted in the propagation of virus for several days. The titer rapidly rises, reaches a peak, then descends quickly. About the 12th day the virus can no longer be found. Soon thereafter specific neutralizing antibody is present in the milk of the quarter which had been instilled. A few days later it may be found in the blood serum and somewhat later in the milk of other quarters of the mammary gland. Because it is not economical to use cattle for experimental purposes, an attempt was made to substitute goats. Trials on these indicated that the virus propagated readily in the half of the mammary gland instilled, that neutralizing antibody formed, and that in general the response of goats was similar to that of cattle. It was observed that there was some difference in the behavior of the immune mechanism following the stimulus brought about by influenza virus and Newcastle disease virus. In the former, antibody rose following propagation, finally leveled off, and with little decline persisted for many months — perhaps might continue to persist for years. In the case of Newcastle disease virus, however, the antibody level was maintained for approximately 5 months when it began to decline and finally almost disappeared. It was, therefore, apparent that the propagation of influenza virus resulted in the development of neutralizing antibody of high level for long periods of time, perhaps permanently, whereas Newcastle disease virus induced a temporary stimulation of antibody production.

One of the interesting features relating to a long term development of antibody is whether a constant antigenic stimulus is necessary or if the rather fleeting experience with the virus is sufficient to maintain production. Because of the difference in the behavior of the host to the two viruses, both of which propagated in the gland, it was felt that the removal of the infected half might throw some light on the site of formation and perhaps the stimulus required for the production of neutralizing antibody. It was, therefore, determined to instill Newcastle disease and influenza viruses into the mammary glands of suitable goats. After approximately 40 days, when the development of antibody was well established, it was planned to remove in one instance the whole gland in the other the instilled half only and determine the effect, if any, on antibody production.

Materials and Methods. — Material used for instillation. — The virus of influenza A (PR 8 strain) was propagated in 9-day-old developing chick embryos. When the embryos were almost dead the eggs were opened and the clear fluids harvested. This usually titrated 10⁷. A dose of 2.0 cc of this fluid was introduced into the lactiferous sinus of the gland.

The Canadian Twist strain of Newcastle disease was employed. It was propagated in 9-day-old embryos. When the embryos died, the clear fluids were collected. This material, which usually titrated 10⁴, was used for instillation.

Methods of instillation. — Healthy goats that were in active lactation were used as experimental animals. The milk was removed from the mammary gland, then by the use of a teat tube and a hypodermic syringe 2 cc of the appropriate clear fluid carrying the virus was instilled into the lactiferous sinus without injury to the tissue. Samples of milk and blood were collected daily and the content of virus determined by means of chick embryo inoculation and the hemagglutination test.

Surgical removal of the gland. — The virus was present in the milk of the half instilled for approximately 10 days after which it disappeared and neutralizing antibodies were soon found present. Approximately 40 days after the instillation of virus and when antibody both in the milk and blood serum had reached a substantial level, the whole gland or that half of
the gland which had been instilled was removed surgically. The appropriate tissues were ground in a colloidal mill and an attempt made to obtain free virus by means of the inoculation of chick embryos and appropriate animals. At no time was free virus found. Fig. 1 to 6 indicate the details concerning the propagation of virus and the titer of antibody before and after the surgical removal of a part or the whole of the gland.

**RESULTS AND DISCUSSION.** — Reference to Fig. 1 and 2 indicate that shortly after the removal of either the whole gland or that half which had been the seat of propagation of Newcastle disease virus there was a precipitous drop in the antibody level of the blood. It is difficult to escape the conclusion that the gland was the principal seat of antibody production and that which was present in the blood was, in large
the gland which had been instilled was removed surgically. The appropriate tissues were ground in a colloid mill and an attempt made to obtain free virus my means of the inoculation of chick embryos and appropriate animals. At no time was free virus found. Fig. 1 to 6 indicate the details concerning the propagation of virus and the titer of antibody before and after the surgical removal of a part or the whole of the gland.

RESULTS AND DISCUSSION. — Reference to Fig. 1 and 2 indicate that shortly after the removal of either the whole gland or that half which had been the seat of propagation of Newcastle disease virus there was a precipitous drop in the antibody level of the blood. It is difficult to escape the conclusion that the gland was the principal seat of antibody production and that which was present in the blood was, in large
measure, an overflow from the gland. The evidence, however, throws little light on the more basic background of whether antibody production is the result of a continuous stimulus or a function of tissue taken on following a short term stimulus. It has been established that once the virus disappeared, free virus could not be found either in the milk or samples of gland tissue which were removed surgically.

The instilling of influenza virus (Fig. 3, 4) brought about a somewhat different result. The blood titer fell following surgical removal of the gland or that part which propagated virus, but nevertheless there continued to be present in the blood for months a substantial level of antibody. It would, therefore, seem that either the production of antibody of the stimulus which brings about the production is not so
Intimately related to mammary gland tissue as in the case of Newcastle disease virus. This is emphasized by the result which followed the instillation of both viruses in the same host (Fig. 5, 6). Following the removal of the gland Newcastle disease antibody fell in 2 weeks to a low level whereas influenza antibody came down slowly and always continued to maintain a substantial level.

The trials which have been described together with those mentioned in a former paper (2) would appear to indicate that the mammary gland is the seat of a substantial production of neutralizing antibody when the virus has been instilled into the lactiferous sinus and propagation taken place. The question of whether a continued stimulus is at work resulting in the formation of neutralizing antibody or whether a
short term stimulus was capable of exciting the immune mechanism for long periods of time is left unanswered. It is difficult to visualize antibody production being maintained at a high level for months in the absence of a continuous stimulus. Some color is given to this view by the fact that following the instillation of both influenza and Newcastle disease viruses the antibody reaches a similar level but only in the influenza virus is this high level permanently maintained. The primary stimulus of each virus which called forth an antibody response does not appear to differ, but since Newcastle disease antibody seems to appear and disappear it suggests that this stimulus is not long sustained and indeed that the falling off of antibody production may be related to the disappearance of continuous stimulus. That virus is not present in a free form does not rule out the possibility that it may be in a state which precludes recognition by available methods.

LITERATURE CITED


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Myxoviruses and Their Propagation in the Mammary Gland of Ruminants

Charles A. Mitchell, Oscar Nordland, and R. V. L. Walker

Gross Ile Experimental Station, Defence Research Board, Department of National Defence; and Animal Pathology Laboratories, Health of Animals Division, Hall, P.Q.

The assistance of Mr. John Wall is acknowledged with thanks.


In 1941 McLelland and Hare (4) and Hirst (2) working independently made the extremely important observation that the virus of influenza agglutinates the erythrocytes of fowl. This laid the groundwork for the technique and extensive use of the hemagglutination method. Others discovered later that the causative agents of Newcastle disease, fowl plague and mumps brought about a similar reaction. Although there are other agents whose products of growth can produce a somewhat similar manifestation, consideration of them can be left out of this communication.

From time to time attempts have been made to classify viruses. The Nomenclature Committee of the Microbiological Conference attempted among other things to establish a nomenclature of these hemagglutinating viruses. This was published in 1955 by Andrews, Bang and Burnet (1) and the four viruses were grouped under the term Myxovirus. The one striking feature common to all is that they agglutinate the erythrocytes of fowl. This note is to direct attention to another common property.

In 1933 Mitchell, Walker and Bannister (5) published the results of attempts to propagate the virus of influenza, Newcastle disease and an unknown agent in the bovine mammary gland. The results suggested that this was possible although no conclusions were drawn. Studies were continued and the results published in other papers (6, 7, 8). This, together with some unpublished work is summarized below:

1) If a small amount of the causative virus of influenza, Newcastle disease or fowl plague is instilled through the teat canal into the mammary gland of cattle or goats, it propagates and virus in the milk reaches a relatively high titer.

2) Propagation continues for about a week when the titer declines and the virus disappears.

3) No evidence of virus in the blood was found.

4) A day or two after the virus disappears neutralizing antibodies are found in the milk; first in the segment which was infected. These antibodies continue for months perhaps for the life of the animal.

5) The gland cannot be reinfected.

6) If the virus has been inactivated antibody formation is not induced, nor do antibodies form when viruses are instilled which do not propagate in the gland.

7) The viruses of Eastern, Western and Venezuelan encephalomyelitis, vaccinia and poliomyelitis (3) did not propagate in the gland.

8) The viruses to which the gland is susceptible are not related to host range; also if introduced by other routes such as subcutaneous, they fail to infect or localize in the gland.

![Graph](image-url)

**Fig. 1.** Hemagglutination titers of milk and blood.
9) No work was done to determine if the specific antibody present in the milk conferred protection in susceptible animals if given by the oral route.

\textit{Myxovirus parotitidis}, the causative agent of mumps and the remaining member of the group behaved somewhat differently and for this reason, a few additional details are given.

\textbf{Material}. — Through the kindness of Dr. F. P. Nagler, Laboratory of Hygiene, Department of Health and Welfare, we obtained vials containing the Ender's strain of mumps virus which had been propagated in chick embryo, the fluids harvested and hophilised. This material was reconstituted with sterile saline solution and used immediately.

\textit{Animal and route}. — A 3-year-old Jersey cow was chosen. It had been in lactation for 10 months and was giving about 28 oz of milk from each quarter. After removing all milk, 8.0 ml of the reconstituted virus was instilled into the lactiferous sinus of the left front quarter through the teat canal, using for the purpose a teat tube attached to a hypodermic syringe.

Except on two occasions when developing chick embryos were used, determination of the presence of virus was made by the hemagglutination test. Samples of milk and blood taken before instillation or a week after instillation and thereafter at intervals until the 33rd day. Blood was also collected at intervals. The results are given in Fig. 1.

\textbf{LITERATURE CITED}


3. \textsc{Kitchin, C. R. Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Ont. Personal communication.}


\textit{NOTE}: Reprints of this article are available without charge. Please circle Reprint No. 3 on the enclosed order form and mail to the publisher. No postage is required.
Preliminary Research on the Prevention of Experimental Poliomyelitis in the Monkey by Injection of Globulin Antibodies Coming from Cow’s Milk

P. Lépine, J. A. Thomas, and J. Leclerc

Pasteur Institute, Departments of Virus Research and Cellular Biology, Paris.


Two of the authors have previously shown that it was possible to obtain in abundant quantity lactoglobulin antibodies of virus. In effect, the cow hyperimmunized against foot-and-mouth disease by inoculations of aphthous virus in the duct of the teat produces a milk rich in specific antibodies. The globulin antibodies precipitated from this milk protected in a significant way the guinea pig and the bovine from experimental hoof-and-mouth disease (3,4).

We prepared lactoglobulin antibodies from the virus of poliomyelitis. The anti-polioimmunization serotherapy formed the subject of numerous experiments, whether it was a matter of the utilization of the sera of convalescents or of anti-polioimmunization sera of animals (1), but in spite of their great interest, the results of this research have not been retained in the usual method for the prevention of this disease. One of us, with Mr. Raynaud and co-workers (2), has shown the possibility of obtaining from the horse anti-polioimmunization sera of a high strength which offers interesting therapeutic prospects.

Given the high titer of globulin antibodies coming from the milk and the abundance of the source, it was logical from these results to apply our method to the virus of poliomyelitis. Does the hyperimmunization of the mammary gland allow for the production of anti-polio- myelitis lactoglobulins of high strength? Cows were subjected to hyperimmunization of the mammary gland in the cattle-sheds of the Institut Pasteur in Paris. The antigen stock came from the Virus Department, which was in charge of standardization and titration of the solutions and experimentation on the monkey. The preparation of the lactoglobulin was carried out by the Department of Cellular Biology.

Immunization of the Cows. — For these first attempts, three milk cows were subjected to mammary hyperimmunization. Two of them received virus attenuated by chemical agents; the third, treated at the very first by a non-attenuated virus, gave the best results. The globulins made use of in the attempt at prevention set forth in this memo came from the milk of this third animal. This cow received an injection of virus, once a week in the duct of each of the four teats of theudder (virus type I, stock 54:1342 cultivated on kidney cells of the monkey, strength 10^4.1, injected dose: 20 ml/teat). The antibodies of milk serum were titered on samples taken 3 days after each injection (seroneutralization on KB cells).

Table 1 summarizes the development of the strength of antibodies in the lacteovirus. It will be noticed that it begins to rise after the third injection (1/40) and the fourth (1/60), and

<table>
<thead>
<tr>
<th>Number of injections preceding the sampling</th>
<th>Date of sampling</th>
<th>Virus type I</th>
<th>Virus type II</th>
<th>Virus type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12 Apr. 1961</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2 Apr. 1961</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>8 Apr. 1961</td>
<td>1/40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>15 Apr. 1961</td>
<td>1/160</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>25 Apr. 1961</td>
<td>1/10,240</td>
<td>1/160</td>
<td>1/10</td>
</tr>
<tr>
<td>Interrupted injections for 2 1/2 months</td>
<td>18 Sept. 1961</td>
<td>1/1,280</td>
<td>1/640</td>
<td>1/320</td>
</tr>
<tr>
<td>5</td>
<td>5 Dec. 1961</td>
<td>1/2,550</td>
<td>1/640</td>
<td>1/40</td>
</tr>
<tr>
<td>6</td>
<td>8 Dec. 1961</td>
<td>1/32,000</td>
<td>1/640</td>
<td>&lt;1/20</td>
</tr>
<tr>
<td>7</td>
<td>15 Dec. 1961</td>
<td>1/16,000</td>
<td>1/640</td>
<td>&lt;1/100</td>
</tr>
<tr>
<td>8</td>
<td>22 Dec. 1961</td>
<td>1/32,000</td>
<td>1/1,280</td>
<td>&lt;1/100</td>
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<tr>
<td>9</td>
<td>19 Feb. 1962</td>
<td>1/16,000</td>
<td>1/1,000</td>
<td>&lt;1/1,000</td>
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<tr>
<td>10</td>
<td>26 Feb. 1962</td>
<td>1/18,000</td>
<td>1/1,000</td>
<td>&lt;1/1,000</td>
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<td>11</td>
<td>9 Feb. 1962</td>
<td>1/8,000</td>
<td>1/1,000</td>
<td>&lt;1/1,000</td>
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<tr>
<td>12</td>
<td>15 Feb. 1962</td>
<td>1/6,000</td>
<td>1/1,000</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>22 Feb. 1962</td>
<td>1/10,240</td>
<td>1/640</td>
<td>0</td>
</tr>
</tbody>
</table>

13
then increases very considerably from the fifth (1/10,240). After an interruption of 10 weeks, the strength falls to 1/1,280. At the resumption of injections, the strength increases to 1/32,000; it falls again then to 1/8,000; this decrease may be due to a slight mastitis which lasted for 3 days, the virus of the eighth injection having revealed itself bacteriologically non-sterile, and also perhaps due to the utilization of a new batch of virus.

In addition, as has been shown in relation to hoof-and-mouth disease (3), the injection of viral antigen via the mammary ducts results in general hyperimmunization; the strength of poliomyelitis antibodies becomes very important in the sanguineous serum.

Preparation and Strength of Lactoglobulin. — The technique of fractional distillation of the milk serum by ammonium sulfate is the same as that previously used (4). The stages of fractional distillation are followed by immunodiffusion in gel (Fig. 1) and by seroneutralization. The strengths of the different fractions are indicated in Table 3. For an appreciation of these strengths, the degree of concentration of the samples in relation to the milk serum must be taken into account; one will see, for example, that the fraction of 0.3 sat. which is concentrated 129 times in relation to the lactosum, gives the elevated titer of 1/160,000, whereas the following fraction 0.4 sat. is much lower in antibodies.

Although the intramammary injections were only given with type I virus, one will note that the seroneutralizing strengths for types II (principally) and III are far from being negligible (Table 3). This is equally true for the blood serum (Table 2). It is a matter here of the phenomenon known as "allurement" due to the common antigens in the three types of polio virus.

Finally antibody activity of these globulins appears stable. Titration of samples of lactosum serum filtered and conserved bacteriologically at 4°C have given the same results at an interval of 11 months.

First Attempts at Prevention in the

Table 2. Antibodies titer of the blood serum after 20 injections of the virus via the mammary gland

<table>
<thead>
<tr>
<th>Blood serum</th>
<th>Date of sampling</th>
<th>Virus type I</th>
<th>Virus type II</th>
<th>Virus type III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21 March 1962</td>
<td>1/256,000</td>
<td>1/10,240</td>
<td>1/40</td>
</tr>
</tbody>
</table>

Fig. 1. Reaction of precipitation in gel, between the virus and the immune lactoglobulins, fractioned by ammonium sulfate, colored by ponceau S. Fractions prepared from raw Cactoglobulins precipitated at 0.5 sat. (fraction I).

a. Fraction II precipitated at 0.25 sat. containing a remainder of casein, relatively unabsorbent antibodies being given the strong concentration of the sample.

b. Fraction III precipitated from the supernatant of II at 0.4 sat., pH 6; concentrated 70.7 times; abundant antibodies.

c. Fraction IV precipitated from III at 0.3 sat., concentrated 129 times; abundant antibodies.

d. Fraction V precipitated from the supernatant of IV at 0.4 sat., concentrated 496 times; antibodies relatively unabsorbant.

v. Poliomyelitis virus type I.

Monkey. — Attenuated. — The subcutaneous injection of 20 cm³ of anti-poliomyelitis purified lactoglobulins produced no reaction in the twenty monkeys thus treated.

Prevention. — The dog-faced baboon was inoculated with a poliomyelitis virus (type I) intracerebrally (0.25 ml to 10⁵). The controls were kept under identical conditions. The treated ones, left in the same cage as the controls, received 20 ml of lactoglobulin antibodies under the skin (titer 1/160,000).

Table 3. Titer of lactoglobulin precipitated by ammonium sulfate

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Vol. milk serum</th>
<th>Date of sampling</th>
<th>Virus type I</th>
<th>Virus type II</th>
<th>Virus type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>III (0.4 sat., pH 6)</td>
<td>70, 7</td>
<td>24 Jan. to 5 Feb. 1962</td>
<td>1/128,000</td>
<td>1/32,000</td>
<td>1/160</td>
</tr>
<tr>
<td>IV (0.3 sat.)</td>
<td>129</td>
<td>4 Jan. to 22 Jan. 1962</td>
<td>1/163,000</td>
<td>1/40,000</td>
<td>1/10,000</td>
</tr>
<tr>
<td>V (0.4 sat.)</td>
<td>496, 1</td>
<td></td>
<td>1/40,960</td>
<td>1/1,280</td>
<td>1/10</td>
</tr>
</tbody>
</table>
In a first trial, bearing on two animals, the controls became afflicted with poliomyelitis on the 14th day and became paralyzed. On the other hand, the monkey having received the lactoglobulin remained definitely unaffected. Another experiment concerned nine monkeys, of which three were controls. The six treated received, respectively, an injection of lactoglobulin antibodies 1 hr before, then 1, 5, 24, 48, and 72 hr after the intracerebral inoculation of virus. A control was overcome by poliomyelitis with total paralysis of the hindquarters on the 14th day; another was overcome on the 16th day with a paralysis less accentuated of the hindquarters; the third resisted spontaneously. Among the six monkeys treated, only the one injected in the 16th hr came down with paralysis on the 14th day, with a total recovery from the 16th day. All the other monkeys remained unaffected.

Commentary. — The first attempts show that the antipoliomyelitic lactoglobulins are perfectly tolerated by the monkey in subcutaneous injection and that they can protect it against experimental poliomyelitis, even when they are injected several hours after an intracerebral inoculation of virus. Additional experiments remain to be done with hypervirulent viruses, and at the same time eventually enlarging the dose of lactoglobulins, in the hope of undertaking experiments of prevention in man.

Literature Cited

NOTE: Reprints of this article are available without charge. Please circle Reprint No. 4 on the enclosed order form and mail to the publisher. No postage is required.
The Action of Specific Lactogammaglobulin in Experimental Poliomyelitis in the Monkey

P. Lépine, J. Thomas, Yvonne Carass, J. Leclerc, Giovanna Ceolin, and P. Sinaret
Pasteur Institute, Paris.

With the technical collaboration of Mr. Louis Chaumont.

The intramammary inoculation of the cow with pathogenic germ (1, 2) for the immunization of the bovine (diathetic immunity) and the production of lactogammaglobulins is applicable to the virus of hoof-and-mouth disease (8, 9): the elevated strength of the antibodies contained in the lactoserum allows by an injection of animals of the same variety, a prophylaxis (prevention) of disease which is particularly effective.

It has been shown (5) that the same method of inoculation allowed the obtainment, parallel to serum antibodies, of lactogammaglobulins (LGG) of an elevated strength against the poliomyelitis virus (which does not determine clinic infection in Bovine), and that these allow for the prevention of experimental poliomyelitis in the monkey. We have attempted to make precise the limits in which a prophylactic action of LGG bovines exercises itself — to compare their action with that of the serum of the horse (7) utilized in analogous conditions and to attempt to arrest the extension of the paralysis once this has been established.

Our attempts have been carried out on baboons (Cynocephalus papio) which received intracerebrally 0.5 ml of a suspension at $10^{-2}$ of medulla of a monkey having succumbed to poliomyelitis; the stock of the virus used (Mahoney stock of type I) normally maintained on the culture of a monkey kidney having undergone the experiment previously several times on the animal in order to confer on it an elevated virulence. Table 1 permits one to examine the validity of the attempt. It shows that under these conditions, the incubation of the disease is from 6 to 11 days. Of 16 control monkeys, seven succumbed before the 6th day of the disease, four showed serious extended paralysis which led to sacrificing them to the agony or at least once their state was stabilized; four survived with after-effects of paralysis; a single one recovered health without after-effects.

An equal number of 16 dog-faced baboons were treated by LGG; among them, 12 received a single injection of 40 ml of LGG having a neutralizing strength of 160,000, at intervals going from 48 to 168 hr. After the injection (table 2). The last four monkeys were already overcome by beginning paralysis when they received the serum and the dose was repeated after 24 hr. They are indicated in Table 3.

Our results call for the following commentary and remarks:

1) The subcutaneous injection of a single dose of 40 ml of LGG of an elevated strength prevents the appearance of paralysis during the period of incubation of the disease provoked by intracerebral inoculation of poliomyelitis in the dog-faced baboon. It is still effective but to a lesser degree and more irregularly in animals presenting premonitory signs of paralysis, to a

<table>
<thead>
<tr>
<th>No.</th>
<th>Duration of incubation and evolution of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Paralyzed the 6th day; dead the 7th day</td>
</tr>
<tr>
<td>2</td>
<td>Paralyzed the 6th day; dead the 9th day</td>
</tr>
<tr>
<td>3</td>
<td>Paralyzed the 7th day; dead the 10th day</td>
</tr>
<tr>
<td>4</td>
<td>Paralyzed the 7th day; dead the 10th day</td>
</tr>
<tr>
<td>5</td>
<td>Paralyzed the 8th day; dead the 9th day</td>
</tr>
<tr>
<td>6</td>
<td>Paralyzed the 8th day; paraplegic the 11th day; quadriplegic the 12th day; sacrificed the 20th day</td>
</tr>
<tr>
<td>7</td>
<td>Paralyzed the 8th day; dead the 13th day</td>
</tr>
<tr>
<td>8</td>
<td>Paralyzed the 9th day; improvement the 14th day; remained paraplegic; sacrificed the 20th day</td>
</tr>
<tr>
<td>9</td>
<td>Paralyzed the 9th day; dead the 11th day</td>
</tr>
<tr>
<td>10</td>
<td>Paralyzed the 10th day; (back left paw); paraplegic the 13th day; sacrificed the 20th day</td>
</tr>
<tr>
<td>11</td>
<td>Nystagmus and trembling the 10th day; incoordination during the following days with partial paralysis back right paw; survived with after-effects</td>
</tr>
<tr>
<td>12</td>
<td>Paralyzed the 11th day; survived with a marked paraplegia in spite of partial recuperation</td>
</tr>
<tr>
<td>13</td>
<td>Paralyzed the 11th day; paralysis at first with progressive extension; sacrificed-agonizing the 18th day</td>
</tr>
<tr>
<td>14</td>
<td>From the 10th to the 16th day; nystagmus, trembling, hesitation about jumping; recovery without after-effects the 16th day</td>
</tr>
<tr>
<td>15</td>
<td>Paralyzed the 11th day; after a form rapidly extensive, recuperated in part but remained totally paraplegic</td>
</tr>
<tr>
<td>16</td>
<td>Paralyzed the 12th day; paraplegic at first with incoordination of the upper members, without improvement on the 20th day</td>
</tr>
</tbody>
</table>

16
Table 2. Monkeys treated preventatively (a single injection of 40 ml LGG)

<table>
<thead>
<tr>
<th>No. of hr before treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>48</td>
</tr>
<tr>
<td>18</td>
<td>48</td>
</tr>
<tr>
<td>19</td>
<td>72</td>
</tr>
<tr>
<td>20</td>
<td>72</td>
</tr>
<tr>
<td>21</td>
<td>96</td>
</tr>
<tr>
<td>22</td>
<td>96</td>
</tr>
<tr>
<td>23</td>
<td>120</td>
</tr>
<tr>
<td>24</td>
<td>120</td>
</tr>
<tr>
<td>25</td>
<td>144</td>
</tr>
<tr>
<td>26</td>
<td>144</td>
</tr>
<tr>
<td>27</td>
<td>168</td>
</tr>
<tr>
<td>28</td>
<td>168</td>
</tr>
</tbody>
</table>

Table 3. Monkeys treated curatively (two injections of 40 ml LGG at 24-hr intervals) from the appearance of the first paralysis

<table>
<thead>
<tr>
<th>No. of treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>8th</td>
</tr>
<tr>
<td>30</td>
<td>9th</td>
</tr>
<tr>
<td>31</td>
<td>10th</td>
</tr>
<tr>
<td>32</td>
<td>11th</td>
</tr>
</tbody>
</table>

stage where half the controls are already paralyzed or dead.

2) This protective action ceases from the appearance of the paralysis; injections of elevated doses of LGG to animals already paralyzed does not modify the course of the disease which evolves appreciably in the treated as well as the controls; at the most it can lengthen somewhat the course.

These findings confirm the earlier conclusions (4). They do not seem to be in accord with the recent research of authors operating with an immunserum obtained in the rabbit (3, 6), of which we until now have not been able to reproduce the results.

3) In comparative trials, in using an immunserum of the horse of an elevated strength, we were not able to assure a protection of the moneky beyond the 40th hr after intracerebral inoculation. Moreover, the serum was poorly tolerated, giving at the place of injection large piacards with inflammatory phenomenon going to the necrosis of the inner lining. It is probable that in this case the failure of late prophylaxis is due to the slowness of the reabsorption of the injected product and to the presence of antacellular antibodies resulting in the necessity in the horse of injecting doses of virus culture close to 100 times more elevated than in the case of intramammary immunination in the cow.

4) In a trial (not reported here) we used instead of bovine LGG, the untreated milk serum of the same animals showing a very high strength in antibodies; the results, although better than that obtained with the serum of the horse, have not been as good as that with LGG, the protection yielding in practice when the injection is deferred by 80 hr. Given the generally admitted identity of antibodies of milk and blood, this apparent contradiction seems to us to result in the fact that the LGG are by their method of extraction subjected to a purification which can, with respect to raw milk serum, facilitate their reabsorption at the point of injection and their diffusion in the organism. The injections of LGG have not provoked hardening or local inflammation. We have not used the way of endovenous administration, effective in the cow (10) but judged contra-indicated in the case of use of a heterologous serum.

Our results confirm the value of the method of intramammary immunization of the bovine for the obtaining of antipoly lactogammaglobulins of a high strength, applicable in the prevention of experimental poliomyelitis in the monkey. They show the effectiveness of the method until the appearance of paralysis, but not beyond this stage. These contestations are of a nature to limit the serotherapy of poliomyelitis to its prophylaxis, to the exclusion of the treatment of the declared paralysis. In the eventuality of the application to man, the question of the tolerance of the bovine lactogamma-
globulins by the human organism was not touched upon.

LITERATURE CITED

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Passive Transfer of Antitumour Factors through Milk in Rats

B. Sekla

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The transfer of antibodies against bacterial or heterologous normal tissue antigens through milk to suckling animals has been repeatedly demonstrated (1). This has not been done, so far as I know, for homologous tumour antigens.

In the experiments reported here, rats 1-2 days old were taken from their normal albino Wistar mothers, and given for nursing to black rats (strain bWf), which are resistant to Walker tumour 256; these were immunized before pregnancy by repeated unsuccessful implantations of the tumour. Other normal Wistar mothers, nursing their own offspring of the same age, were used as controls.

All sucklings in an experimental group were inoculated, subcutaneously in the supracapular region, with equal, relatively low doses of live tumour cells (200,000 or 100,000) in 0.1 ml saline. Precautions were taken to ensure the strictest possible equality of the doses in every litter in a given experiment, by shaking the suspension in the container and in the syringe, and by inoculating rats from each of the three litters in turn. In all experiments of this kind normal Wistar sucklings were, to a certain extent, protected against the growth of the tumour inoculum by the milk of the immune bWf nurse. The differences between individual experimental sets in the degree of protection can most probably be explained by quantitative variations, for example, the number of tumour cells used or differences in the immune state (titer) of the bWf nurses.

As an example, results of two such experimental sets are summarized in Table 1.

With the doses used, no new tumours appeared, as a rule, after the 20th day following inoculation, which was performed on the second or third day of life. First tumours usually appeared from the 10th day onward. Deaths of young animals with tumours occurred regularly in the 7-10 days following. Occasionally, a Wistar or a bWf suckling, either protected or unprotected by the intake of immune milk, died from rapidly developing lung metastases of the tumour, even when the primary tumour at the site of implantation was very small or invisible. Here a direct inoculation of tumour cells into the blood stream of the suckling may be suspected.

The offspring of the bWf immune or normal mothers, even when nursed by a normal Wistar female, may resist tumour growth in varying degrees, usually depending on the dose of tumour cell used. Their resistance may be explained most plausibly by the relatively long lag in tumour growth with small inocula, so that the young bWf rats have time to produce tumour antibodies of their own. In the offspring of immune mothers, some passive transfer of antitumour factors by way of placenta, or intake of immune milk (colostrum) in the short interval before the exchange cannot be excluded.

Another experiment, on "double passive transfer," was undertaken. The offspring, all born within an interval of 36 hr, of three normal Wistar females, were inoculated simultaneously with a dose of about 250,000 live tumour cells each, and left to nurse with their own mothers. One of the mothers was injected intraperitoneally, after delivery, with relatively large doses of immune rat serum (4 ml every third day, about 20 ml altogether); the second mother rat was injected with the same dose of normal rat serum, and the third was left untreated. The immune serum was taken, this time, not from bWf immune rats, but from resistant Wistar rats, which can be selected from our commercial stock.

On the 18th day after tumour inoculation, the following proportions of palpable tumours

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Tumour Strain of</th>
<th>Nurse Strain of</th>
<th>Tumour No. suckling</th>
<th>Tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 X 10^5 (a) bWf</td>
<td>Wistar normal, 5/11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>immune from (b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>Wistar normal, 3/7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>Wistar Own offspring, 9/9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10^5 (a) bWf</td>
<td>Wistar normal, 0/7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a)</td>
<td>immune from (b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>Wistar normal, 0/7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>Wistar Own offspring, 8/8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Ratio of the number of sucklings showing palpable tumours on the 20th day from implantation to the total number of the litter.

It appears here that the antitumour factors from the immune serum injections, although diluted by the metabolic stream of the recipient mother, are still effective in tumour inhibition in the sucklings. But conditions were different: the tumour dose was relatively high; the last serum injection to the mother was done on the ninth day after tumour inoculation, so that by the 18th day its effect may have been exhausted; lastly, the milk proteins and antibodies are more thoroughly digested by the maturing enzymes of the sucklings (a). Therefore, during the following 10 days, tumours also appeared in all but one of the sucklings in the protected litter, so that the protection was manifested here only by a postponement of tumour growth.

Besides these, a “feeding experiment” was also undertaken. Sucklings nursed by their own normal Wistar mother were given daily, by means of a pipette, some drops of either immune or normal rat serum by mouth. Here, too, there was an indication of protection against tumour growth.

In the experiments on “milk protection” it was noted repeatedly that a normal (non-immune) bWf' nurse cannot protect sucklings against the tumour growth.

The results of these experiments agree remarkably with results of passive transfer of antitumour factors by serum injections (2, 4), or by injections of culture media from tumour-immune spleens (3). They are very persuasive evidence that these antitumour factors circulate, very much as do the antibodies of current immunology.

Participation of a general transplantation immunity component in these results cannot be wholly excluded, but the relative share of this factor may be rather small: Wistar sucklings nursed by a bWf' female, immunized by a tumour of Wistar origin, grew normally, and only their tumour growths were inhibited. The influence of this anti-strain effect of immune serum may be even less in the double transfer experiment, where immune serum of Wistar rats was injected into Wistar females nursing their own offspring.

LITERATURE CITED

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Diathetic Immunization of Sheep against a Rat Cancer
B. Sekla and E. Holečková

Department of Biology, Medical Faculty, Charles University, and Laboratory of Metabolism, Czechoslovak Academy of Sciences, respectively, Prague II, Albertov 4.


The term “diathetic immunization” has been introduced by B. Campbell et al. (1), who instilled different antigens into the orifice of the milk gland (in cows) and obtained colostrum and milk with high titers of antibodies against the antigens used. They consider the milk gland as an exocrine reticuloendothelial gland.

It has been shown by previous work that resistant rats (strain bW, or black-Walker-resistant, inbred in our laboratory) produce, after implantation of the Walker 256 tumour, factors inhibiting the growth of this tumour in susceptible rats, when resistant immune blood serum is injected into them intraperitoneally (3), and that susceptible rat sucklings are protected against the growth of the tumour by the ingested milk when nursed by the resistant immune rat females (4).

Further, it has been shown that spleens of resistant rats immunised by the tumour in vivo continue the production of antitumour factors when explanted in tissue culture (2), and that an active immunization in vitro of spleens from normal resistant rats against the tumour used is possible (6).

In the present experiments, we instilled repeatedly, at weekly intervals, by means of a glass canule, several million of live Walker 256 tumour cells suspended in about 0.5 ml of saline into the orifice of the milk glands of two sheep a month or more after parturition. Instillation was carried out on the evening after the last (third) day's milking. Twelve hr later (on the next morning) the milking was continued.

There was a massive cellular reaction in the milk from the instilled udders, changing gradually, in the course of the immunization, from nearly exclusively polymorphonuclear (segmented) leucocytes to more and more mononuclear cells with a large plasmatic component. (This cellular reaction will be described in detail and analyzed in a separate communication.) There was also a strong decline in the milk output beginning at 18 hr after instillation, but in the following 3-5 days milk output returned nearly to normal. There were no signs of bacterial inflammation of the instilled udders or of the animals themselves, although some capsulated bacteria have been seen in cell preparations from the milk; milking was done under the usual non-aseptic conditions. The cellular reaction as well as the inhibition of lactation seem to vary from individual to individual; both were much stronger in one sheep than in the second.

The “immune” milk was used in several ways:
1) A neutralization test was performed. Rats were implanted with equal doses of the Walker 256 tumour cells, which had been incubated for 3 hr at 37°C in the normal and immune milk (only the milk fat was separated by centrifugation). After incubation, the cells were centrifuged from the milk and resuspended in saline before implantation. A control set of rats was implanted with non-incubated tumour cells (from the same original suspension) in saline. Results are given in Table 1.

2) Milk serum was prepared by adding some drops of dairy rennet and centrifuging the curdled milk, and injected after Seitz-filtration intraperitoneally into susceptible rats bearing implants of equal numbers of live Walker 256 cells. Results of two groups of 10 male rats weighing 120-150 g at the start of the experiment, and given, from the day of tumour implantation, 2 ml daily of normal or immune milk serum for 40 days, are illustrated in Fig. 1.

![Fig. 1. Deaths from tumour in rats injected with normal and immune milk serum.](image-url)
Table 1.

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7/10 (70%)</td>
</tr>
<tr>
<td>In normal milk</td>
<td>17/20 (85%)</td>
</tr>
<tr>
<td>In immune milk</td>
<td>9/20 (45%)</td>
</tr>
</tbody>
</table>

*Ratios of rats with tumours to the total number of animals used; after 50 days no new tumour appeared.

Table 2.

<table>
<thead>
<tr>
<th>Injections</th>
<th>Mean gain in weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>16%</td>
</tr>
<tr>
<td>Normal milk serum</td>
<td>15</td>
</tr>
<tr>
<td>Immune milk serum</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 3.

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>No. of immunizations</th>
<th>before inoculation</th>
<th>after inoculation</th>
<th>6 days Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>100</td>
<td>96</td>
<td>82-100</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>100</td>
<td>57</td>
<td>48-67</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>100</td>
<td>18</td>
<td>10-28</td>
</tr>
</tbody>
</table>

In the normal series there are 2 non-takes in 10, in the immune series 5 non-takes in 10.

It is to be noted that the gains in weight of rats given the immune milk serum were lower than those given the normal milk serum or of the controls. The gains in weight of different groups after 15 days from the beginning of the experiment are given in Table 2.

After 15 days, the weight control is obscured by the growing tumours. On the whole, the anti-rat component of the heterologous (sheep) immune milk under given conditions seems negligible; all rats surviving without tumours, both in the experimental as well as in the control groups, seem undamaged and live normally to date.

3) Cytotoxic effect of the immune milk serum was proved by the method of unstained cell count [3]. Freshly prepared tumour cells were suspended in normal and immune milk serum, counted, incubated at 37°C for 4 hr and counted again. Table 3 indicates results of these counts on subsequent days, where the influence of milk sera from three different sheep after different numbers of immunizations are compared. In all these experiments, drops of complement were added (guinea pig serum), but we have found that fresh immune milk serum has a cytotoxic activity of equal degree without the addition of complement.

These results, considered with previous work, seem to prove that any system capable of immune reaction is able to produce antitumour factors passively transmissible, and active in inhibiting tumour growth in susceptible animals. Furthermore, there seems to be a possibility of producing relatively large amounts of these active substances by a procedure which is very simple and obviously harmless for the producing animal. Thus some kind of immunotherapy could be used in combination with other therapeutic means in cancer.

Attempts to separate the active substances from the milk serum (as milk globulins), as well as to purify them from the anti-species component, are proceeding. Also the possible role of the cellular milk reaction and the eventual use of such cells are being considered.

Literature Cited


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Immune Milk Treatment of Rheumatoid Arthritis—Review
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HISTORY. — One of the most important discoveries of the 20th Century is the relationship of bacterial infection and hyperimmunization to rheumatoid arthritis.

In the 1930's and 1940's, Edward C. Rosenow (10), Mayo Clinic, and M. Wetherby (15), University of Minnesota Medical School, conducted experiments which led to the isolation of Streptococcus and Staphylococcus from patients with rheumatoid arthritis.

In the very recent period there has been researched by Mayo staff members contributing to our knowledge of bacteria and arthritis (13). Also, during this period, Sir William E. Petersen et al. (8, 12) did research at the University of Minnesota concerning bacterial immunization of animals for the production of antiserum for disease control. Particularly important were the studies dealing with the immunization of the bovine and the finding that milk serum would give relief to patients with rheumatoid arthritis.

The importance of the agents in causing the individual to develop immunity and the specific hypersensitivity of the individual are widely recognized. Luther L. Terry (14), Surgeon General, Public Health Director, stated the importance of the immunological aspects to the prevention and cure of rheumatoid arthritis at a meeting of the Committee of Appropriations of the House of Representatives.

BASIC PRINCIPLES. — Milk serum from hyperimmunized cows contains high levels of antibodies. When a person ingests these antibodies, the univalent (small blocking) antibodies protect the individual. The person receives help in two ways: (i) the antibodies are carried to the site of infection to assist the body defense, and (ii) the antibodies move to the site of the reactive surfaces where the antigen-antibody complexes are and result in a neutralization-type action.

We believe that arthritis is the result of deposition of an insoluble antigen-antibody complex of the bacteria in the connective tissues of the patient, such as reported by Felton (3), with the accompanying development of collagen. The foci of infection provoke a low-intensity allergic inflammation which is the essential nature of the arthritis. These sites of allergic reaction are generally the joints and articular surfaces, just as the sites for allergic reaction against inhalant

antigens (pollens) tend to be centralized in the nasal and upper respiratory tract.

The importance of bacteria to hyperimmunization of rheumatoid arthritis is based on extensive studies (2). Dawson (4) has shown that serum from patients with rheumatoid arthritis agglutinates and precipitates hemolytic streptococci. Sixty-seven % of the cases of rheumatoid arthritis gave agglutination, whereas other workers showed positive results in 84% of the cases. Paul Holbrook (6, p. 22), Tucson Arthritis Clinic, says, "Occasionally, rheumatoid arthritis of acute onset cannot immediately be differentiated with certainty from rheumatic fever." It is generally accepted that the hemolytic streptococci, Group A, are responsible for rheumatic fever.

Streptococcus viridans has been isolated from the foci of infection in many cases of arthritis (9, 15). Staphylococci were isolated from patients and are thought to be a primary cause of arthritis as cited by Crowe (3). Diplococcus pneumoniae has been recognized as a causative agent since reported in 1914 (13).

The antigen used in the hyperimmunization of the cows is based upon the importance of these agents. The antigen is a suspension of killed organisms in a physiological saline solution. Each cubic centimeter contains Staphylococcus aureus, 4,000 million; Streptococcus viridans, 4,000 million; Streptococcus hemolyticus, Group A, Types 1, 3, 4, 5, 6, 12, 13, 16, 49, 4,000 million of each; and Diplococcus pneumoniae, 4,000 million. This antigen is specifically compounded for use in the continental United States by the North American Antigen Company, St. Paul, Minnesota.

The absorption of immune bodies (gamma globulin) through the intestinal tract of adult humans,, root from milk by Klemperer (7) and from serum by Burrows and Havens (1), has been substantiated by Petersen and Campbell (8) and used as a means of treatment of disease as reported by Raabe (9).

SPECIFIC CASE STUDIES. — Two cases in the early experimental work supervised by B. Campbell, University of Minnesota Medical School, will illustrate some of the findings. The cows producing this milk were hyperimmunized by the author.

Case 1. — The patient being observed was a female, 36 years of age, who was diagnosed
as having rheumatoid arthritis in the hands and wrists. She was using salicylate for pain relief. The right wrist was so weakened she could no longer turn a door knob, nor could she wash dishes. She began drinking the milk on 3 September 1955 and within 4 days had partial remission. Within 7 days she had complete remission but continued on the milk for 2 months. Her hands and wrists strengthened, and as of 1963 the remission has been permanent.

Case II. — The subject, a female of 53 years, had arthritis generally dispersed in the lower back and legs, particularly in the knees. She started drinking milk at the rate of 1 quart (1 pint at a time) daily on 26 November 1957. On 2 December she noticed that her pain was disappearing, and it required 27 days for full pain remission. She continued drinking the milk until 23 December 1957. On the following 5 January she noticed that the symptoms were returning, and by 13 January her condition was that of the beginning of the experiments. (This may indicate that there was only sufficient immunity to overwhelm temporarily the reactive surfaces.) Beginning 6 April 1958, milk was again taken at the same rate. First she noticed relief of pain, and by 11 April there was complete relief of symptoms. The ingestion of milk was continued until 25 June. There has been no return of arthritis.

Large Scale Experiments. — Our findings from analyzing the records of persons drinking 1 quart of immune milk/day indicate that over 50% of those persons having joint involvement, diagnosed by medical doctors as rheumatoid arthritis, benefited. However, the percentage of those who improved decreased to below 60% when persons with all types of joint involvement were considered.

Cyril M. Smith (11), Minnesota physician, conducted a large sample survey of 199 persons who used immune milk in the treatment of rheumatoid arthritis symptoms. Smith reported that immune milk was successful in 56.8% of cases reported. This improvement occurred within 3 months. The greatest improvement was noted between the second and fourth weeks. However, in some cases it required more than 6 weeks before a marked improvement was noticed.

The tabulation of results revealed that the immune milk was more frequently successful in those cases that had responded to aspirin prior to taking the milk than in those that had not.

Twenty-three % of 113 persons who found relief from symptoms while taking milk experienced an increase in pain prior to their improvement. The great majority of the persons who experienced this pain made marked improvement.

Smith (11) found that “Steady consumption of the milk as directed results in relief with fewer quarts of milk than if intake is not steady.”

Literature Cited

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The Problem of Immune Milk and the Gamma Globulin Fraction as Examined in Its Role in Lymphatic Allergic Conditions in Children and Adults with Special Disorders of Rheumatoid Arthritis

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The lymphatic child often seen by the physician in his practice also shows this child to be afflicted with some focal infection. In many cases the child is brought by the mother because his schoolwork has become successively worse and his concentration poorer. In spite of the positive motivation on the part of the child, no improvement is noticed although previously his schoolwork was of good quality. Upon examination, a typical adenoid lymphatic condition is found besides the typical facial expression. When the mother or older child, and also the adult, is questioned the following is ascertained: there is a predominately left-sided occipital neurology, a neurology of the plexus brachialis and the nerve centers of the arm, often looked at falsely as metabolic disturbances. There is a pounding pain in the region of the 5th to the 7th vertebrae of the neck, the 7th to the 9th thoracic vertebrae, the 2nd to the 5th lumbar vertebrae, and into the intercostal spaces, as well as “organ sensations,” e.g., arthritis of the shoulder joints. In 1955 Raabe (22, 23) described this “half-sided neuritis” with all its symptoms and consequences as a secondary infection. He claimed that heart attacks or heart trouble proved to be secondary intercostal neuritis, stomach trouble ziphoidal neurology, that pains in the lower abdomen have their origin in the lumbar region, and that occipital neurology causes serious psychic, mental, and physical disturbances. The child makes errors of carelessness, is not able to concentrate, is called lazy, etc., and no one looks to the tonsils as the cause. The adult complains increasingly of dizziness, disturbance of the equilibrium, headaches, and finally pressure in the heart region and in the stomach; sleeplessness and depression bring on thoughts of suicide.

The patient is examined at the clinic and is often returned to his physician as “healed” with the diagnosis vegetative “Dystony” which is in reality, for example, a sinusitis maxilaris. After discontinuing the sedatives and the symptomatic medication, the general practitioner must get his patient well, and therefore he occupies himself without any essential aids more with the symptomatic factors and finds his success within the frame of a para-neural-novocain-caffeine treatment. He succeeds with this treatment of occipital neurology in so far that the patient feels relaxed and for a short transitional time he has again his old powers of concentration and his physical well being. The relaxation and the normalization of the metabolism (Organübertätigkeit) achieved through the novocain-caffeine treatment proves to the doctor his diagnosis. Thus novocaine serves to establish the diagnosis; it is a means to an end. The healing factor lies to an extent in the caffeine as was established in the many comparative experiments done in series by the author himself (1947, 1948). We also know of the relaxing effect of the novocain therapy from Schleich and Speransky and Aslan as well as from other numerous authors and champions of novocain. However, causally seen, every event (issue) must have a cause. We forget today (1962) that the basically gifted child suddenly becoming poor in concentration represents the beginning of adult troubles, the inception of the exogenous and constitutional infection, a propensity toward infections (12). Already in the child, and according to my own observation from the fourth year on, one must look for the beginning of the primary chronic rheumatism of the joints and other diseases. “The lymphatic child is a particularly easy prey to illness. He is inclined toward catarrhal infections of the upper respiratory tracts; colds, angina, bronchitis succeed one another. The lymphatic tissue is hypertrophied, because the body is continually on the defense against disease. This defense reaction is, however, disturbed and imbalanced” (31, 32). Then tonsillectomies are performed without strict indication in more cases than can be estimated. The focal infection is indiscriminately removed and every conservative attempt is neglected (omitted). On the other hand, so little attention is paid to the lymphatic components of the local infection and every indication pointing to tonsillectomy is omitted, and thereby the ground is laid for early invalidism of millions of people.

With this in mind attention must also be called to basic views and work of Pfaundler (19), Saller (26), Zikh (32), Maas (12), H. E. Wolf (31), and others. The conservative
therapy (e.g., Lymphozil) which supports the following discussion of the treatment with immune milk must also be pointed out. The problem of immune milk makes it necessary to do away not only with the aftermath of local infections, but also remove the causes of rheumatism, lymphatic allergic conditions, asthma, skin diseases, etc.

The most basic work of former times has been forgotten. L. Briege and Paul Ehrlich's (2) work "About the Transmission of Immunity through Milk." The goal of the investigation is to achieve immunity, to normalize the unstable defense reaction and recognize the causal factors in their inception. This was already the goal of Paul Ehrlich's work. Petersen and Campbell (17) attacked this problem anew in 1955 in view of the untold numbers of people invalidated through allergic asthma, rheumatic conditions, and other illnesses. Though Behring, Kitasato, and others had thoroughly treated the problem of active and passive immunity, Campbell, Petersen, Porser, and Porterfield (3, 4, 17, 20, 21) returned to the problem. In exhaustive experiments they proved that one can enrich milk with specific antibodies if the cows have been treated with specific antigens during the dry period. They used a great number of antigens such as phenol-killed bacteria, virus, pollen, and tissues from other species, simple albumens, and others; they infused this material in known concentrations directly into the teats (cisterns) and the milk channels of the udder. This infusion was carried out weekly for 4 to 5 weeks before parturition. It was shown that the milk contained a considerable amount of specific antibodies at about the 10th day of lactation. Furthermore, it was established that these antibodies were mainly in the gamma globulin fraction of the milk. The immunization bodies are in the strongest concentration during the colostrum milk period since the colostrum milk is particularly rich in globulins (27).

Normally the cow produces only antibodies against its own species. Since we do not know the specific antibodies naturally present in the colostrum, these antibodies are designated as nonspecific (27).

The possibility to stimulate the udder of cows or goats to form specific antibodies by mammary infusion of antigens was established in extensive research studies by Mitchell and his co-workers (13, 14), Struss (29), Seelmann (25), Lasilila and Senft (11). Senft and Porter (28) could further ascertain that it is possible to maintain the production of specific antibodies over a longer lactation period by a 4- as well as a 10-day infusion interval. On the basis of the extensive results of these experiments, the milk gland is considered as an exocrine reticular endothelial system in which the production of specific antibodies can be artificially stimulated.

Senft (27) has already reported on the treatment of the animals, the production of immune milk, and the isolation of the globulin fraction with high concentration of specific antibodies.

Campbell and Petersen immunized cows against grass pollen and gave the milk to pollen-sensitive people. They observed that in the pollen-sensitive period of the year the pollen test became negative. The allergic symptoms decreased more and more, first the asthma, then colds, and finally the itching of the eyes disappeared.

Critical observations on the problem of allergic research and the use of human gamma globulin were made by Sir Henry Dale (6), Parrot (15), Laborde (10), Benda (1), Urquia (30), F. Schefthart et al. (24), Gillissen (8), and others. The formation, the storing, the pharmacetical efficacy, the biological activation and elimination of shock poisons, e.g., shock substances, were examined. They were able to prove that during analytical and allergic reactions certain shock poisons were freed. The histamine to which Sir Henry Dale and co-workers (6) ascribed the role of the most important mediator of allergic reaction lost for a time its first place, because other shock substances were discovered. Parrot, Laborde, Benda, Urquia, and others supposed that the blood of allergics lacked certain substances which counteract the histamine effects and called this histaminophyox. The reduction (agglutination?) and inactivation in the blood was called histaminopexy. According to the French authors the latter is almost exclusively controlled by the globulin fraction which travels electrophoretically with the gamma globulin. Other serum globulin also shows a certain histaminopexy capacity.

Allergics have a clearly reduced histaminophyox, e.g., histaminopexy, according to Parrot (15) and Laborde (10). They supposed that histaminophyox could be increased by the formation of antibodies through active immunization with a gamma globulin-histamine complex (histaglobin) and that the histamine is considered as a hapten. The histamine which is later freed during an antigen-antibody reaction should then be biologically inactivated by an antihistamine-antibody reaction. Research with guinea pigs oriented in this direction demonstrated that after several applications of
histaglobin complex, the guinea pigs survived a histamine dosage which 3 weeks before would have been fatal to them. Favorable results were also recorded in the clinic in treating allergic bronchial asthma, vasomotor rhinitis, urticaria (hives, and migraine. Gillis and Fillipek (8) investigated the possibility of defining a differential diagnosis and therapy. They came to the conclusion that by giving a parenteral dosage of a complex of histamine and gamma globulin, particularly in asthma, an antihistamine immunity occurs. They noted a clinical rate of success in the treatment of bronchial asthma of 35% to 65%. The ready-made product is Histadestal; it is used subcutaneously. Histadestal is a complex mixture of exactly defined gamma globulin of human retroplacental serum of unstable (endständig) carbohydrates. It is particularly important that the relation of 12 mg immune globulin: 0.09506 gamma histamine is guaranteed (warranted).

Through a kind of active immunization, Histadestal leads to the formation of specific antihistamine antibodies and also influences other shock poisons. Personal observation with only single dosages of Histadestal were successful from 8 weeks to half a year with vasomotor rhinitis, allergic asthma, and other idiosyncrasies. In one case when the dosage was repeated an improvement of vasomotor rhinitis was achieved for more than half a year.

The production of human globulin from retroplacental serum is not only interesting in view of gamma globulin fraction from milk of lactating animals, but must lead to a co-ordination of research with animals and the clinical fields.

Petersen and Campbell (18) showed within the framework of treatment of rheumatism of the joints with human beings that they could effect a reduction of pain and better mobility with 14 out of 17 volunteers. In one case Campbell and Petersen concluded that after initial recovery several relapses were caused because the treatment was discontinued. They observed in four other cases seemingly complete recoveries where treatment was uninterrupted. The final conclusion was that in rheumatic diseases there are deposits of insoluble antigens of Streptococcus in the tissue and joints. They cite a similar observation by Felton (7) with pneumococci polysaccharides. They prove that the antigen is the real cause of the inflammation of the joints and the allergic reaction. The immune milk contains materials against this antigen and by continued use causes the antigen deposits to disappear and the tissue to heal. However, one must insist that the experiments could not be carried out sufficiently long. In a further detailed paper, "Antibodies in Milk as Protection against Human Diseases," Campbell and Petersen discuss explicitly and clearly the methods of experimentation and the immune milk problem.

The task of this paper is to place at the disposal of critical science the following case. The permission of the female patient was obtained.

**Case History. Patient:** female, Marie S., born 17 May 1900.

**Grandparents, female line:** Grandfather, 1845-1925; Grandmother, 1848 - 1911. Grandfather from age 20 to 65 had crippled legs. Grandmother was in bed 9 years with crippled joints, knee-creep position. From this marriage, four boys and two girls. Oldest son only moderate rheumatic complaints; oldest girl from age 14 to 75 had serious joint and limb deformities and had to be fed; other two daughters healthy.

**Grandparents, male line:** 1844-1927 and 1849-1909. Three healthy sons; one daughter who for 25 years had serious rheumatic changes of joints and had to be fed.

**Parents:** Father, 1873-1927; Mother, 1874-1909. One son, one brother of the patient, had arthritic deformation of the spinal column and the large joints; one daughter (the patient).

**Early history of the patient:** As a child, measles, chickenpox, diptheria; at age 16 tuberculosis of the lungs; at 22 right-sided pleuro-sy, at 27, 29, 31, and 42 normal births. At 33 displaced uterus, at 47 possible heart embolism. Had always had rheumatic complaints and many headaches and nervous pains. In 1950, jaundice, two gall operations with consequent permanent liver damage and heart muscle decompensation with ecema and blocking of the liver ducts. Myocarditis was continually treated to the year 1962. In 1954 the bones on the base of the skull and neck were X-ray treated, because of intolerable pain (headaches) and therapy resistance to anti-rheumatic and para-neural novocain therapy. In the summer of 1954, sciatica on the left side (11 April to 18 September). Twenty-five para-neurology treatments were necessary and at the same time also strophantin treatments were given. In 1956, access in the right groin. (In order to realize the serious heart condition it is noted that the patient received about 300 strophantin injections from 1954 to 1959. Only after that could the digitalization be accepted as adequate.) In January 1957 and March 1957, double-sided bronchial influenza; in 1957, the first suspicion of cirrhosis of the liver with chronic liver blockage and heptitis. In October 1957, influenza...
with bronchitis; in April 1958, ascites (not on account of the cirrhosis of the liver but as a consequence of the heart decompensation). After September 1960, diffuse skin bleeding Morbus Werlhoff on both legs possibly because of thrombocytopenia or some toxic rheumatic changes.

Present complaints: Continual pains, hands always swollen, joints are painful. Can no longer comb her hair, or do housework, cannot use knife and fork. Her face is always swollen because of constant decorinin treatment which swells her whole body (Cushing syndrome). All other rheuma medication she cannot tolerate because of the liver. She has not noticed any amelioration of her condition.

Findings: 61-year-old woman, well nourished, moderate physical strength. Blood circulation in skin and visible mucous membrane good. Head: Wears bifocals; Ear: hearing capacity both sides 6 m, both ear drums w. f. (without findings); Mouth: false teeth; Tongue: not enlarged, w. f. Neck: No goiter, no swollen glands. Chest: symmetrical, shallow breathing, both sides vesicular breath sound; Head: resonance full. Lungs: limits of ex- and inhalation mobile. Heart: spread one-fourth of a finger to right and left, the tip can be felt in the fifth intercostal space, systolic noise (murmur?) above mitralis, diastolic noise over the tricuspidal. Beat 68/72 per min. Abdomen: Lower edge of liver by one-half finger wider than rib-vaulting (cavity, costal arch). Epigastric region free, other parts of abdomen free, both kidney regions not sensitive to touch or pressure. Genitals w. f., rectal examination w. i. Nervous system: Reflexes, Romberg's and Babinski's phenomenon negative. Pain upon pressure in the peripheral nerves especially at the emergence of the occipital nerve, major right and left in the plexus brachialis region, the intercostal nerves from the 4-8 at Proc. xiph., the ischias nerve and its branches. Extremities and joints: Generalized serious arthritic changes in the smaller and medium joints, beginning of the stiffening process mainly in the hands and fingers and the joint in the right foot. Inflamed rheumatic swelling of various types in all joints, particularly in the joint of the right foot and leg. Veins form nodules from the size of a pin to the size of a pea (lentil). The left leg shows much the same picture. The patient can hardly move the large and small joints without pain and after a night's rest there are many swellings in the joints. Vertebra: Soreness to the touch in the 5-7 cervical, 5-10 thoracic, 5-5 lumbar vertebrae and in the lower back.

Diagnosis: Primary chronic degenerative Poly-arthritis rheumatica, spondylitis. Spondylitis of the vertebra column, the large joints of the vertebra and nerves controlled by the spinal column. (Neurology in the region of the occipital nerves of the plexus brachialis, the nerves of the arms, intercostal neurology, ischias neurology) Myodegeneratio cordis decompensation. Hypostasis. Conditions after choles-tectomy toxic rheumatic vascular damage, variousis symptom complex. During the treatment from 1946 to 14 May 1961, the following medications were used: salicylic preparations: impletol and others, igtapyrin, butazolidin, deltabutazolidin, elestol, rosin, chloroquin, implanine (placenta extract), and from 1958 decorinin (prednison); daily also from 4-6 pain-killing tablets. Up to 14 May there were signs of Cushing's disease and prednison therapy was used. Because of resistance to other therapy the hormone therapy was the only means to alleviate the condition of the patient.

Technical data: Hypostasis reaction according to Westergreen: 16 April 1960, (1) 35/60; 16 May 1961, 35/60; 12 June 1961, 49/60; 5 July 1961, 77/113; 14 December 1961, 45/85. The blood picture shows 21% lymphocytes and 76% neutrophiles, a partially toxic granulation of neutrophiles can also be noticed. Takata-Ara reaction (Manke-Sommer): 60 ng % (findings of 20 April 1960). Electrophoresis of 25 April 1960: Albumin = 52%; a 1 = 2.5%; a 2 = 6%; β = 11.5%, γ = 28%, other 6.3%. Takata-Ara 60 mg %. Increase of gamma globulins. Electrophoresis of 16 May 1961 by a Takata-Ara of 70 mg %. Albumin = 53%; a 1 = 4.6%; a 2 = 9.0%; β = 11.4%; γ = 22%, other, 7.4%. Red blood picture of 31 July 1961: Hemoglobin color hypochromic; pochilocytosis, none; megalocytosis, none; polychromes, normal; anisocytosis, none; megaloblasts, none. White blood picture of 31 July 1961: Eosinophiles, 7%; Basophilic neutrophiles, 2%; neutrophiles segment, 67%; lymphocytes, 21%; monocytes, 3%. Blood culture of 4 August 1961: Pseudomonas aeruginosa (pyoceaneum). Electrophoresis of 10 January 1962: Albumin = 51%; a 1 = 4.0%; a 2 = 7.6%; β = 11.4%; γ = 26%; other, 8.5%.

Methods of experiment: For the production of immune milk, goats 4 to 5 weeks before parturition were used. They were treated by intramammary method, weekly with 5 ml (2.5 ml for each half udder) of a Staphylo-Serobacterin vaccine. The milk was collected daily and the gamma fraction isolated. The
globulin fraction was made into powder by lysophilization. The goats were repeatedly treated (intramammary) after the 10th day of lactation in order to stimulate the production of specific antibodies during the entire period of lactation. In order not to expose the dry milk to fermentation, it was put up in gelatin capsules which dissolved in the small intestines. The dosage of each capsule was 150 mg/capsule. Treatment began on 15 May 1961, with 54 capsules at 150 mg = 8.1 g and a daily dosage of 2 x 150 mg. On 12 June 1961, i.e., after 28 days, there was a rise of hypostasis reaction from 30/60 (before the experiment) to 45/60; after 25 days more a rise to 77/115, in a total of 51 days. Though milk was only given to 11 June 1961, there was a noticeable rise of the hypostasis up to 5 July, a sign of a specific defense reaction. This rise was mistakenly construed as a sign of infection, and 4 g of antibiotics (broad spectrum) were administered. Thus, the first reaction from the 13th to the 18th day, with its concomitant of pain in the joints and the rise of the hypostasis as a defense reaction, was not correctly evaluated or understood. This was later found out. After the 2 g of antibiotics (broad spectrum), the hypostasis sank on 11 July to 35/60; after a repeat dosage of 2 g on 18 July the hypostasis was 30/60 (the same as before the treatment). A repeated control on 9 August showed again 30/60. The patient complained from the 10th to the 13th day and from the 16th to the 19th day of increased pain and swelling of the joints. But from the 19th day on there was a steady improvement of the mobility of the large and especially the small joints of the hands, though the deformation was still discernible. During the period of the first treatment, the patient took no anti-rheumatic medication or hormones. She used only pain-diminishing remedies. After giving the first 8.1 g = 54 capsules at 150 mg from 15 May to 12 June, she received again until 5 July 46 capsules at 150 mg = 46 capsules at 150 mg = 7 g; thus, twice daily 150 mg. The hypostasis on 5 July was the highest observed.

It was shown conclusively that after a seeming relapse from the 10th to the 13th day and from the 16th to the 18th day, a subjective as well as an objective recovery was achieved. That the clearly rising hypostasis was an expression of the immunization process was proved upon continuation of the treatment.

On 15 September 1961, the treatment was continued with a dosage of three times 150 mg immune milk powder/day for 7 days, then two times 150 mg as a daily dosage; in all 52 capsules = 7.8 g were given. To repeat: from 15 September to 21 September, three times 150 mg; from 22 September to 6 October, 2 times 150 mg; then from 12 November to 19 December 1961, again three times 150 mg; then for 7 days from 5 December 1961 two times 150 mg. This is a total of 8.1 g. From 15 May to 15 December 1961 the patient had received 31 g of immune dried goats milk powder in capsules soluble in the small intestines. This is possibly 1.7% globulin content.

There was no particular pain reaction or other discomfort from now on. The hypostasis showed no noticeable increase with the one taken on 14 December which was 45/65, the initial 16 May 1961 and the one of 16 April 1960 with 35/60, even when considering a simultaneous inflammation of the gall bladder (choleangitis) as well as a chronic interstitial hepatitis, e.g., hepatitis. The symptoms of the joints were also subjectively and objectively decidedly better. The photographs (see Fig. 1, 2) do not show the actual success, such as mobility, etc. A decided improvement may be seen in the region of the right ankle joint. The patient was not able to comb her hair or peel potatoes in the beginning and also could not do without hormones or many pain-alleiating preparations. Now at the close of the present treatment, 5 December, and during the present treatment she managed with only pain-alleiating tablets and a few vibration massages. With no other method to combat rheumatism, whether medicinal or balsamologic, has such convincing success been achieved. At the same time this treatment is very protective to the internal organs and structures.

The healing effect of this method is not to be doubted. There is also a blocking of the degenerative arthritic processes. The body is no longer burdened with anti-rheumatic preparations, particularly hormones and a Cushing's disease with its consequences. Light housework is facilitated by the mobility of hand and finger.

![Fig. 1. Patient Marie S. on 15 May 1961 at beginning of treatments.](image-url)
that here is a revolutionary case. It needs urgent clinical research so as not to miss the possibility to save immense sums of money in the future. Early invalidism of rheumatism and allergies is very costly. And there might be a chance to help those who suffer from sclerosis.

Hochrein (9) in his book *Rheumatic Diseases, Origins and Treatment* published a few very interesting numbers which have economical and social significance. He writes that in 1949, 7.5% of all illness (inability to work, absenteeism) was caused by rheumatism (arthritis) as well as 6.1% of the total days of sick leave. A third of the cases and days due to heart diseases must be counted also, so that nearly 8% of sick leave is on account of rheumatic diseases. In a Krankenkasse (state health insurance) which has more than 2.5 million members there were 50,000 cases of illness and 1.2 million days of absenteeism due to rheumatic diseases. If the whole area of the German Republic were considered, one would have 400,000 cases, a loss of 9.2 million days of work, and 27,000 people incapacitated by this disease. To these numbers one would have to add the army and the pensioners. In 1950 Hochrein writes that 11.5% people become invalids because of rheumatism, only 5.4% on account of tuberculosis. In the months from April to December 1950 there were 3,069 men and 8,919 women invalided because of this disease. However, the social significance lies in that fact that all ages are attacked from child to adult.

All research for a drastic causal therapy including the time-consuming and in the end unsatisfactory chloroquin treatment is unimportant in view of the possibilities of treatment with immune milk which can be used even in hopeless cases. This treatment creates a broad field of new possibilities if science, industry, agriculture, and the state exploit this chance.

Finally it must be expressly stated that in this work only one case is described, since at this time it is not possible to produce immune milk in greater quantities. Later experiments are planned as a series. Furthermore the following is noted:

1) The proof that rheumatic diseases are caused by deposits of insoluble antigens of *Streptococcus* tissue in joints that are attacked must be brought by Campbell and Petersen or by autopsies done with this in mind.

2) The question of reabsorption of immune bodies needs necessarily a clinical proof in order to establish what changes take place in the blood titer. Therapy could then be more exact.
3) X-ray findings were not included, because the case here submitted is in the collegien state and the joints do not yet show the deformation which is seen in the last stage, although there was already very limited mobility of the joints.

4) The purpose and intent of this work is the description of an experiment which was successfully undertaken by a general practitioner. It shall be noted that more research is needed, for rheumatism is a pressing social problem of the first order with which the country doctor is closely acquainted. Intensive research is, however, only possible under certain circumstances and they are not found in the overloaded practice of a country doctor. But the clinic must not look down upon these researches without examination. This may be touching a hot iron, but it is done with intention to provoke the proper places to continue this work.

LITERATURE CITED


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Suggestions to Authors

The basic guide for authors is presented in the Style Manual for Biological Journals, American Institute of Biological Sciences, 2000 P Street, N.W., Washington 6, D. C.

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2. Manuscripts should be double spaced.
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