(Table I) may be summarized as follows: Irradiation with 3,000 r units does not produce permanent damage of the cell culture; with few exceptions, cultures exposed to this dose survive and after several passages show a quite normal rate of growth. Irradiation with 4,000 r units produces variable results; out of 17 cultures treated with 4,000 r units, 11 cultures succumbed in the course of the succeeding transfers, while 6 cultures survived. Irradiation with 5,000 r units renders prolonged cultivation of the cell colonies impossible. A dose of 5,000 r units is, therefore, to be regarded as the minimal "delayed lethal dose" for chicken fibroblasts.

14211

## Effect of Biotin Deficiency on Duration of Infection with *Trypanosoma lewisi* in the Rat.*

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Before August, 1942, when this study was undertaken, there had been few reports on the effect of a vitamin deficiency on the course of a parasitic protozoan infection. Since much work on biotin deficiency had already been done in this laboratory this vitamin was thought suitable for the purpose. It was planned, therefore, to determine the effect of biotin deficiency on the course of infection with a blood protozoan.

*Trypanosoma lewisi* was chosen because it was considered advisable to use an organism whose course of infection had already been well studied.[1]

The rats used in these experiments were white albinos bred for the most part in this laboratory. The experimental rats were kept on a modified egg white diet consisting of:

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| Cornstarch | 185 |
| Salt mixture | 50 |
| Peanut oil | 150 |
| Cod liver oil | 30 |
| Water | 25 |
| Egg albumen† | 300 |
| Sugar | 185 |

They were also given the following vitamin supplement daily: thiamine, 20 γ; pyridoxine, 20 γ; riboflavin, 25 γ; calcium pantothenate, 100 γ. In addition the experimental rats were given 50 mg of choline chloride thrice weekly. The food intake was high in all cases until the terminal phases of the biotin deficiency. The control rats were kept on a modified Sherman diet,[2] with liver or beef twice a week and cabbage once a week in addition.

All rats in a specific group were inoculated intraperitoneally with the same number of *Trypanosoma lewisi.*‡ However, the numbers of parasites in these inocula were only roughly approximate from experiment to experiment. Examination of the blood, tail blood or if necessary, heart blood, was carried out every other day until the end of the infection. After the first disappearance of the parasites from the blood the rats were followed for 6 days and then observations were discontinued. It should be noted that these rats were not *Bartonella* free, but since this was true of both the experimentals and the controls it was felt that the differences in the 2 groups can be safely ascribed to the differences in their diet.

* Under a grant from the Rockefeller Foundation.

1 Taliaferro, W. H., "The Immunology of Parasitic Protozoa" in *Protozoa in Biological Research*, edited by G. N. Calkins and F. M. Summers, Columbia University Press, 1941.

† Purchased from the Weideman Co., Cleveland, Ohio.

2 Smith, A. H., and Bing, F. C., *J. Nutrition*, 1928, **1**.

‡ Our strain of *Trypanosoma lewisi* was supplied us through the courtesy of Dr. W. H. Taliaferro of the University of Chicago.

*Experimental Studies.* Group I consisted of animals in an advanced stage of biotin deficiency used in the preliminary experiments. These animals either had survived to the end of their infection or to 30 days after inoculation. The controls were inoculated over the same period of time and had been intended for controls in the groups which were subsequently amalgamated. Due to circumstances beyond our control at this time the normal rats were not selected as to age and weight to be the equivalents of the experimental rats. The 10 experimental rats had a mean length of infection of 34.9 ± 2.97 days based on the following individual lengths of infection: 14, 30, 30, 33, 35, 37, 38, 40, 43, 49 days. The 13 control rats had infections of 7, 8, 9, 9, 10, 13, 13, 21, 24, 24, 26, 28, 28 days respectively which gave a mean length of infection of 16.9 ± 2.3 days. The value of $t$ for this group was 4.874 > 2,831 for 21°'s of freedom, P = .01§

The rats with the biotin deficiency thus showed a significantly longer course of infection than the normal rats. However, due to the inadequacy of the controls a similar series was repeated.

In Experiment II 9 rats with moderate to advanced symptoms of biotin deficiency were used.‖ Four of these died before the 20th day and are not used in the data.¶ Five rats of the same age which had been kept on the Sherman diet were used as controls.

The 5 biotin deficient rats had infections of 35, 39, 39, 39, 41 days in length which resulted in a mean length of 38.6 ± 1 day. The 5 control rats had a mean length of infection of 21.8 ± 1.5 days based on individual infections of 19, 20, 20, 25, 25 days in duration. The value for $t$ in this experiment was 9.38 > 3.355 for 8°'s of freedom, P = .01. These results in which the controls are adequate definitely confirm those of Group I. Rats exhibiting moderate to severe symptoms of biotin deficiency have a significantly longer course of infection than the normal rats.

Experiment III was carried out in order to determine whether the same delay in termination of the infection would be seen in rats showing only slight symptoms of biotin deficiency. Two groups of rats were used in the experiment. The first group was composed of 5 rats** from the same lot as those used in Experiment II and the controls were normal rats of the same group. The second group was composed of 7 slightly larger rats†† which showed about the same symptoms of biotin deficiency and its controls were of approximately the same age. The same amount of inoculum was given intraperitoneally to both groups and their controls.

The lengths of infections in the 8 surviving experimental rats were 31, 34, 36, 36, 37, 37, 37, 41 days respectively which resulted in a mean length of infection of 36.12 ±1. The 4 controls had a mean length of infection of 22.5 ± .5 days based on infections of 22, 22, 22, 24 days in duration. The value of $t$ for Experiment III was 9.06 > 3.169 for 10°'s of freedom, P = .01. Therefore, the rats showing slight degrees of biotin deficiency had as great a delay in terminating their infections, compared to the normal rats, as did the rats showing moderately severe symptoms.

Experiment IV was devised to see whether rats having latent biotin deficiency would also have a prolonged infection. A litter of 9 rats was divided into 2 groups. The first group of 6 rats was fed the egg-white diet. The remaining 3 were put on the Sherman diet. They were infected as soon as the majority of the experimental rats were beginning to show initial symptoms of biotin deficiency.

In this experiment the 6 experimental rats had infections of 25, 27, 27, 31, 37, 45 days in length which resulted in a mean length of infection of 32 ± 3.1 days. The 3 control rats had a mean length of infection of 17.3 ± 2.6 days based on individual infections of

§ The authors are indebted for aid in statistical analysis to Dr. Norman C. Wetzel, Babies and Childrens Hospital, and the Department of Pediatrics, School of Medicine, Western Reserve University.

‖ One had slight symptoms of biotin deficiency and subsequently improved.

¶ Six other rats with farther advanced symptoms were inoculated at the same time but all of them died before the 20th day.

** Three died.

†† One died.

12, 20, 20 days respectively. The value for $t$ in this case was $3.0002 < 3.499$ for $P = .01$. However, $t$ was $3.000 > 2.998$ for the value of $P = .02$. Thus the difference between the controls and the experimental rats was not as significant and clear cut as in the preceding experiments. The value for $P = .02$, on the other hand, indicates there was a slightly significant prolongation of infection. This experimental group, as might have been expected, can, therefore, be said to occupy an intermediate position between normal rats and those showing definite symptoms of biotin deficiency.

A greater dispersion as to length of infection occurred in this group. The heaviest rat in the group, with a weight of 120 g at the beginning of the infection, became negative first, in 25 days; the lightest rat, with a weight of 92 g at the beginning of the infection, became negative last, in 45 days. A further point of interest was that the experimental rats showed no increase in their slight symptoms of biotin deficiency while they were infected with *T. lewisi*. Even more interesting is the fact that the experimental rats began to show signs of the deficiency in almost the exact order in which they became negative.

Experiment V was done to determine whether the prolongation of infection in the biotin-deficient rats was due specifically to biotin or to the lack of some other element in the diet. Litter-mates from 2 litters born on the same day were used in the next experiment. Five rats from one litter and 3 rats from the other were placed on the deficient diet,‡‡ while 3 rats from the first litter and 2 rats from the second were given the Sherman diet.§§ When slight to moderate symptoms of biotin deficiency appeared in all the experimental rats they were infected with *Trypanosoma lewisi*. Each deficient rat was given .5 $\gamma$ of biotin subcutaneously daily, beginning at the time of inoculation and continuing until the infection was terminated. The 4 surviving deficient rats which had been treated with biotin had infections of 14, 26, 28, and 29 days in length respectively which resulted in a mean length of infection of $24.25 \pm 3.46$ days. Their 4 controls had a mean length of infection of $21 \pm 2.6$ days resulting from individual infections of 17, 17, 22, 28 days respectively. There was no significant difference between the biotin-deficient rats and their controls.

Since there was no significant difference between the 23 biotin-deficient rats of Experiments I to III, for purpose of comparison they were amalgamated and their mean length of infection, $36.13 \pm 1.35$ days, determined. The data from all the 29 normal rats in all 5 experiments was similarly treated and their mean length of infection $19.8 \pm 1.2$ determined. Since the 6 latent deficient rats occupied an intermediate position they were not included in computation of the mean of the biotin-deficient rats.

Comparing the 4 deficient rats which had been treated with biotin during their course of infection with the 29 normals from all 5 experiments, it was seen that there was no significant difference between the mean lengths of the infections $24.25 \pm 3.46$ and $19.8 \pm 1.2$. The value for $t$ in this case is $1.25 < 2.57$ for $P = .01$ for 31°'s of freedom. A similar comparison with the latent biotin-deficient rats with a mean length of infection of $32 \pm 3.1$ days showed no significant difference between them and the deficient rats treated with biotin which had a mean length of $24.25 \pm 3.46$ days; $t$ is $1.62 < 3.35$ for 8°'s of freedom, $P = .01$ in this case. However, the slight difference that occurs is toward the normal side. While a comparison of the 23 biotin-deficient rats, with a mean length of infection of $36.13 \pm 1.35$ days, with the 4 biotin-deficient rats which had been treated with biotin, having a mean length of infection of $24.25 \pm 3.46$ days, showed a definite and significant difference. $t$ in this case had a value of $5.46 > 2.785$ for 25°'s of freedom, $P = .01$.

*Discussion.* From the above data it seems conclusive that biotin deficiency results in great prolongation of infection with *T. lewisi* in the rat. This effect does not seem dependent on the severity of the symptoms as long as the symptoms are definite. Latent biotin deficiency also results in prolongation but of less significant degree. Treatment of

‡‡ Four of which died.

§§ One of which died.

the deficient rats with biotin results in shortening of the lengths of their infections to a point where they are not significantly different from the normals.

With regard to the lack of appearance of further symptoms noted in the latent biotin-deficient rats of Experiment IV, it should be noted that a number of the moderately biotin-deficient rats able to withstand the added strain of infection with *T. lewisi*, had already given the impression of improvement of their deficiency symptoms at or shortly after the height of infection. These phenomena led to the hypothesis that the parasites might be capable of producing sufficient biotin to prevent much aggravation of the deficiency symptoms of their host. Experiments are now in progress to definitely determine whether there is such an effect.

The effect of biotin deficiency in prolonging the infection with *Trypanosoma lewisi* in the rat makes Trager's recent paper[3] indicating a similar lengthening of infection in avian malaria by means of the same deficiency more interesting. This is especially so because the same effect was noted in the Sporozoa as is here noted in Mastigophora.

Studies of the stained material are now being worked out in detail to determine the effects of biotin deficiency on the parasite numbers and on the antibody sequence. Further work is also being done to determine the effect of excess biotin on the infection in the normal rat. It should be noted that since this work was done exclusively with biotin deficiency no claims are made at present that such an effect is specific to biotin.

*Summary.* Biotin deficiency has been found to prolong the infection with *T. lewisi* in the rat. This effect can be negated by the administration of biotin to the deficient rat during the course of infection. Biotin appears to be instrumental directly or indirectly in the activation of the immune bodies in this infection.

3 Trager, W., *Science*, 1943, **97**, 206.

## 14212 P

## Production of Rh Antiserum by Inoculation of Guinea Pigs with Human Erythrocytes.

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Antisera used for detecting the presence of the Rh antigen in human erythrocytes have been of two types: the serum of guinea pigs immunized against the red blood cells of *Rhesus* monkeys,[1,2] and the serum of certain Rh— human beings who have developed antibodies against the Rh antigen.[3,4] These latter sera are not readily procurable since human beings possessing the Rh antibody are infrequently encountered and accordingly the incidence of their employment is greatly restricted. Guinea pig anti-rhesus sera also have limitations to their usefulness. Certain differences have been noted in the Rh antigen as found in monkey and human erythrocytes,[5,6] and the implications of these differences as yet have not been clarified. Further, the production of such sera in certain laboratories may be hampered by the present war-time scarcity of *Rhesus* monkeys. For these practical reasons, as well as for certain theoretical con-

1 Landsteiner, K., and Wiener, A. S., *J. Exp. Med.*, 1941, **74**, 309.

2 Gallagher, Fred W., and Jones, Lloyd R., *J. Immunology*, 1943, **46**, 9.

3 Levine, P., Katzin, E. M., and Burnham, L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 346.

4 Wiener, A. S., and Forer, S., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 215.

5 Davidsohn, I., and Toharsky, B., *Am. J. Clin. Path.*, 1942, **12**, 434.

6 Fisk, Roy T., and Foord, Alvin G., *Am. J. Clin. Path.*, 1942, **12**, 545.