## AD NUMBER

**AD023933**

## NEW LIMITATION CHANGE

TO

**Approved for public release, distribution unlimited**

FROM

**Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Dec 1953. Other requests shall be referred to Office of Naval Research, Arlington, VA 22217.**

## AUTHORITY

**Office of Naval Research ltr dtd 13 Sep 1977**

Office of Naval Research

Contract No. Nonr-811(02)

Rate: First Year $4630.00
Second Year 4100.00

Institution: University of Tennessee
Knoxville, Tennessee
Department of Bacteriology

Title:
A Study of the Influence of Bacterium tularensis on the Amino Acid Metabolism of Animal Hosts.

Period covered in this report:

January 1, 1953 to December 31, 1953

Signed: John M. Woodward
John M. Woodward
Project Director

ABSTRACT OF RESULTS

A. Since start of project:

Chromatographic analysis of the blood of normal rats and rats infected with Bacterium tularense has shown that during infection there is a pronounced decrease of 12 free amino acids in the blood of the infected animals.

Chromatographic analysis of the urine of normal and infected animals has demonstrated the presence, in low concentration, of seven to nine amino acids. No significant differences could be detected in the number or concentration of these compounds in either the normal or infected picture.

Preliminary studies also have shown that there is a pronounced decrease of non-protein nitrogen and a slight decrease of urea nitrogen in the blood of infected animals as compared to blood samples for normal rats.

B. During current report period:

Further studies have confirmed the preliminary observation that the non-protein nitrogen and urea nitrogen levels are depleted in the blood of infected rats as compared to normal animal .

By other clinical blood tests it has been possible to show that the total serum protein also is depleted in infected animals, and nearly all tularemic rats exhibit a moderate hypoglycemia. Furthermore, evidence that liver dysfunction

occurs is provided by tests which demonstrated that the ictoric index was abnormally high in infected rats, many of which also gave a direct test for bilirubin in the blood.

No significant hematological changes occur during infection, but rats recovered from tularemia exhibit a leucocyte count which is nearly twice as high as that found in both normal rats, and in animals during the course of the infection.

Biochemical tests of blood from recovered rats which were reinfected with Bacterium tularense demonstrated that the clinical picture was identical to that of normal animals, thus emphasizing the high degree of immunity to tularemia acquired by rats that have survived the infection.

Administration of amino acids by intraperitoneal inoculation during infection has no significant effect on the average day of death or on total deaths due to tularemia. No difference was observed in the average day of death or on total deaths among three groups of tularemic rats maintained on a protein deficient, normal, or protein enriched diet, respectively.

## PLANS FOR THE FUTURE

A. Immediate:

Studies are now in progress in an attempt to determine the effect of Bacterium tularense on the metabolic activity of of the liver. Investigations of amino acid oxidase activity,

and the utilization of glucose and Tricarboxylic acid cycle compounds by liver homogenates with the Warburg technique will be continued from the present time until termination of this contract August 31, 1954.

B. Long Range:

A similar investigation should be made using animals which are highly susceptible, and which build up little or no immunity to tularemia. Laboratory animals such as the guinea pig or rabbit would be ideal for such a study. It is quite likely that a comparison of the biochemistry of the disease in susceptible animals with that of the relatively resistant rat would shed new light on the mechanisms by which Bacterium tularense exerts a damaging influence in its hosts, and also would be valuable in determining steps to be taken in the management of the disease.

The following constitutes a report on work done under Navy Contract Nonr-811(02) from January 1, 1953 to December 31, 1953.

## PERSONNEL

Research Assistants

Mr. Anthony J. Sbarra, B.S. 1948, Siena College, Londonville, New York
A.B. 1949, University of Indiana, Bloomington, Indiana
M.S. 1951, University of Kentucky Lexington, Kentucky
Candidate for Ph.D. degree. Started work September 1, 1952. Resigned September 30, 1953 having completed all requirements for the Ph.D. degree except final doctoral examinations.

Mrs. Mary Williamson Mayhew, A.B. 195[illegible]. University of Kansas, Lawrence, Kansas
Candidate for the Master of Science degree. Started work October 1, 1953.

Animal Care

Mr. Richard Ross was employed on an hourly basis for feeding and care of our animals until October 1, 1953.

Mr. William Smith replaced Mr. Ross from October 1, to December 12, 1953.

Mr. William Medley was employed as a replacement for Mr. Smith January 1, 1954.

Preventive Immunization

Mrs. Mayhew gives a positive skin reaction when tested with Dr. Foshay's skin test vaccine. Since she worked with B. tularense while at Kansas it is possible that she may have contracted an inapparent infection at that time. We do not consider it necessary, or desirable, to proceed with her vaccination.

## SUPPLIES AND EQUIPMENT

Supplies: Once again, the major purchases have been made for rats. Early in the year we obtained rats weighing 180 - 200 grams for 95 cents each. More recently we have been able to buy Wistar rats from a local source for 75 cents apiece. In addition, the University of Tennessee Department of Animal Husbandry has supplied us with more than 200 surplus rats free of charge. These have enabled us to extend our studies to good advantage.

Other supplies have been obtained from the Bacteriology Department with the exception of a Micro-Waring Blender which was purchased for the preparation of normal and infected rat tissue homogenates for Warburg studies.

## TECHNICAL REPORT

As stated in our first report, the chief object of the work described in this paper has been a study of the effect of Bacterium tularense on the amino acid metabolism of rats infected with this organism. In the work previously reported we observed a disturbance of amino acid balance in the blood of tularemic rats. The major aim of subsequent research has been an attempt to determine the fate of the free amino acids shown to be depleted in the blood during infection by techniques described below. Also, we have made subsidiary investigations of the clinical blood picture such as complete blood counts, icteric index and blood glucose levels in normal, infected, recovered and in reinfected recovered rats.

A limited investigation of nutrition with relation to tularemia in rats was also conducted.

## GENERAL METHODS

Techniques described in our previous report will not be duplicated here.

**Clinical blood and urine tests:**

Blood Non-Protein Nitrogen

The Blood Non-Protein Nitrogen (hereafter to be referred to as NPN) was determined by the method of Folin an Wu (1919).

Blood Urea Nitrogen

For the determination of the Blood Urea Nitrogen (hereafter to be referred to as BUN) the method of Karr (1924) was employed.

Urine Urea Nitrogen

The urine urea nitrogen was determined by the method described in the Manual of Clinical Laboratory Methods by Hepler (1951).

Blood Glucose

Blood Glucose levels were determined by the Method of Folin and Wu (1920).

Icteric Index

The method described in Practical Physiological Chemistry by Hawk, Oser, and Summerson (1948) was employed for the determination of the Icteric Index.

Van den Bergh

The Van den Bergh test for bilirubin was conducted using the method of Van den Bergh (1921).

Total Serum Protein

The method of Greenberg (1924) was used for the determination of the total serum protein.

Hematology

Oxalated blood, collected as previously described was used for all hematological determinations except for the leucocyte differential count, in which whole blood was used.

Hemoglobin Concentration

The Hemoglobin concentration was determined by the method of Karr and Clark (1941).

Erythrocyte and Leucocyte Counts

The Erythrocyte and Leucocyte counts (hereafter to be referred to as RBC and WBC respectively) were performed as described in the Manual of Clinical Laboratory Methods by Hoplor (1951).

Differential Leucocyte Count

The leucocytes were differentiated in accordance with the classification of Schilling as reported in the Manual of Clinical Laboratory Methods by Hoplor (1951).

Nutritional Studies

This phase of the work was divided into two parts, the first part dealt with the administration of a mixture of amino acids and also of cystoine and arginine singly by an intraperitoneal route to rats infected with tularemia. Three daily injections were given each rat of either the amino acid mixture or the individual amino acid. The mixture of amino acids was composed of those previously identified in the serum of the normal rat. The concentration of each amino acid was 1 mg. per ml.

The second portion of this work was concerned with a group of rats fed ad libitum on diets containing 5, 20, and 30 per cent

protein for a period of 21 days. The composition of the diets are as follows:

| | Per cent Protein | | |
|---|---|---|---|
| | 5 | 20 | 30 |
| Wesson Oil | 5 | 5 | 5 |
| Pearl Corn Starch | 86.2 | 67.45 | 55.0 |
| Casein | 4.5 | 23.25 | 33.75 |
| Mineral Mixture | 5. | 5. | 5. |
| Yeast (Brewers) | 3. | 3. | 3. |
| Choline chloride | 0.1 | 0.1 | 0.1 |
| Vitamin A (stabilized) 6000A | 0.1 | 0.1 | 0.1 |
| Vitamin D (stabilized) 2000D | 0.1 | 0.1 | 0.1 |

Per 30 Kilos

| | |
|---|---|
| Riboflavin | 100 mg. |
| Vitamin $B_1$ | 50 mg. |
| Vitamin $B_6$ | 50 mg. |
| Pantothenic Acid | 100 mg. |
| Folic Acid | 50 mg. |
| Vitamin $B_{12}$ | 1 mg. |
| Vitamin K | 5 mg. |
| Para Amino Benzoic Acid | 100 mg. |

## EXPERIMENTAL RESULTS

### Clinical Studies of Blood and Urine in Normal Rats and in Rats Infected with Bacterium tularense

Observations of the decrease in the free amino acid level in the blood of rats infected with tularemia suggested an investigation of the effect of the infection on the clinical blood and urine picture of the normal rat as compared to the rat infected with Bacterium tularense.

### Non Protein Nitrogen and Blood Urea Nitrogen Levels

Several experiments were conducted using a total of 38 normal and 37 infected rats for the determinations of blood NPN levels. A total of 46 normal and 33 infected rats were used for the determinations of the BUN levels. The BUN and NPN determinations were made simultaneously on the blood sample from each normal and infected rat. The results of these determinations are presented in Tables 1 and 2.

It is evident that there is a definite decrease in the NPN level in the infected animals. There is also a statistically significant decrease in the BUN level of the infected animals.

Total Serum Protein Levels

Since a disturbance of the NPN and BUN levels has been observed, it seemed of interest to determine what effect the infection had on the total serum protein. A group of 10 normal rats and 10 infected rats were used. The results of this experiment are shown in Table 4. A decrease in the serum protein of the infected rat is evidenced.

Urine Urea Nitrogen

In an attempt to determine if an accelerated protein metabolism occurs during infection, as evidenced by an increased urine urea nitrogen, an analysis was made of the urine of 27 normal rats and 30 infected rats. Table 3 shows the results of these investigations. Apparently, there is no significant difference in the urine urea nitrogen of the normal rat as compared to the infected rat.

Blood Glucose Levels

A decrease in the free amino acids in the blood accompanied by no increase of these compounds in the urine conceivably may be attributed to a deamination. Keto analogues, or glucose synthesized from these moieties possibly might be found in the blood of infected animals. A comparison of the glucose levels in the blood of infected animals with those of normal animals might be indicative of this type of synthesis as evidenced by a hyperglucemia. Experiments involving 28 normal rats and 40 infected rats were performed. However, the data presented in Table 5 indicates that a hypoglycemia resulted.

Liver Function Test

Since the organisms tend to localize in the liver, it is possible that liver dysfunction results.

Consequently, an experiment was devised in which 45 normal and 34 infected rats were used. Results tabulated in Table 6 indicate that the Icteric Index was elevated in the infected rats. The Van den Bergh tests were positive with a "direct reaction" from 15 of the 34 infected rats, in contrast with a negative reaction in all of the normal rats as shown in Table 7.

Hematology

A secondary anemia has been reported in tularemia in humans by Pullen and Stuart (1945). Liver dysfunction as evidenced by an increased serum bilirubin indicated that a hematological investigation of the infected rats might be profitable. The results of such studies are recorded in Table 8. A total of 10 rats were used. No appreciable hematological change was observed in the infected rats.

A Study of the Clinical Blood and Urine Picture in Recovered Rats and in Those Reinfected with Bacterium tularense

Previous results demonstrated that reinfection with the Sm strain does not alter the concentration of free amino acids in the blood of animals recovered from the disease. It seemed logical that no abnormal blood chemistry would be detected in the reinfected rat. The blood of reinfected animals should be very similar if not identical to that of the recovered rat. Accordingly, blood chemistry determinations were made on recovered and reinfected rats. Groups of 10 rats were used for NPN, BUN, serum protein, Icteric Index,

Van den Bergh and Blood Sugar Determinations. Results are recorded in Tables 9 to 14. In no instance was there a statistically significant difference in the blood chemistry of recovered rats and those reinfected.

Chemistry of the Urine of Recovered and Reinfected Rats

Urine Urea Nitrogen levels in recovered and reinfected rats were determined. No significant change in these levels occurred in the reinfected rats, as is indicated in Table 15.

Hematology of Recovered and Reinfected Rats

Complete blood counts were performed on the blood of recovered and reinfected rats. Once again no significant hematological change was noted in reinfected animals, as shown in Table 16.

Nutritional Studies

Since it has been shown that the free amino acid level in the infected rat is depleted, treatment with a mixture of the amino acids found in the free state in the blood of the normal rat seemed advisable.

In addition, the effect of infecting animals maintained on a 5, 20, and 30 per cent protein levels was assayed. Table 17 lists the results of experiments directed toward ascertaining the effect of injecting the rats with a mixture of amino acids or some single amino acid at the time of infecting and 2 daily intraperitoneal injections of the amino acid misture or cysteine and arginine alone as compared with the control group given physiological saline and subjected to no treatment.

Table 18 contains the results of experiments involving animals maintained on various levels of dietary protein. No significant difference in the average day of death was observed, this despite the fact that animals maintained on a 5 per cent protein regimen lost approximately 10 grams; those provided with 20 per cent protein maintained their weight; those given 30 per cent protein diets gained approximately 5 grams each.

## DISCUSSION

Previously we reported a depletion of free amino acids in the blood of rats infected with Bacterium tularense. Several explanations for this phenomenon may be considered. It seems possible that since the organisms tend to localize in the liver and spleen they may utilize the free amino acids directly from the blood as it is filtered through these organs. This utilization may account for the depletion of these compounds during infection. The fact that cystine, which is an essential metabolite for Bacterium tularense, disappears from the blood of infected animals lends further support to the suggestion that the organisms utilize the free amino acids of the host.

It is possible, also, that the organisms exert a damaging effect on the liver in such a manner as to stimulate enzymatic activity in the degradation of these compounds. In either instance, it follows logically that the free amino acid level of the blood would be disturbed. Consideration also must be given to the concept that, during

infection, there may be interference with other metabolic activities such as cellular respiratory functions of the liver.

In an attempt to elucidate this "depletion mechanism" attention was first focused on the non-protein nitrogen and blood and urine urea nitrogens levels of the normal rat and of the infected rat. The term, non-protein nitrogen, includes the nitrogen of the blood which is not precipitated by the usual precipitating agents. It is a heterogenous mixture of compounds including urea, ammonia, amino acids, creatine and creatinine, as well as other nitrogenous substances which are usually spoken of as an undetermined nitrogen. They represent intermediary products of protein metabolism in the process of transportation, or end products of metabolism en route to excretion.

Knowledge of the levels of the NPN, BUN, and UUN may, therefore, serve as an indication of the state of protein metabolism. The results indicated that the NPN levels of the infected rats were lower than in the normal rats. The difference is of statistical significance as evidenced by the 90 per cent confidence interval of the difference of the mean, which rejects the null hypothesis that $\bar{X} - \bar{X}' = o$ ($\bar{X}$ = mean of normal, $\bar{X}'$ = mean of infected). Since the free amino acid level in the serum of the rat was shown to be depleted during infection, this result was not unexpected. The decrease in the NPN level may be attributed, at least in part, to a corresponding decrease in the free amino acid level.

It is quite conceivable that deamination of the amino acids in the liver of the infected rat is accelerated, and that the deaminated residues are converted to carbohydrate, ketone bodies and other com-

pounds in preparation for dissimilation at a higher rate than in the liver of the normal rat. If an accelerated deamination occurs it could be expected that an increased BUN and UUN would follow due to an increase in ammonia which is then converted to urea.

The BUN levels of infected rats were found to be slightly lower than those of normal animals. These data indicate that the depleted amino acids are probably not converted to urea. The UUN levels of the infected rats are not significantly different from those of the normal rats. These findings suggest that there is no apparent increase in tissue catabolism.

Since amino acids constitute the "building stones" for the synthesis of protein, it appeared advantageous to determine the effect the infection has on the total serum protein of the host. Our results show that the infected rats had lower total serum protein level than did normal animals.

If there is an accelerated deamination it follows logically that the deaminated residues may be synthesized to glucose, and thus a hyperglycemia might be expected. Apparently this is not the case, since a hypoglycemia was observed in infected animals. The ability of the liver to store glycogen may be reduced, or the keto analogues may be used directly by the organisms in the liver. Possibly both mechanisms are involved.

The data thus far obtained indicate that the organisms themselves may utilize the amino acids and/or the keto acids. Support for this hypothesis may be derived from the chromatographic analyses previously reported.

Cystine, an essential metabolite for the organism, frequently was missing completely from chromatograms of the serum of infected rats. It appears that the organism prefers to use for its food supply easily accessible, low molecular weight compounds, in preference to the more complex proteins of the host. Since digestive processes would be involved requiring much energy for protein breakdown, the organisms probably prefer the path of least resistance such as assimilation of the smaller compounds.

Since the organisms tend to localize in the liver a disturbance of liver function might be expected. The Icteric Indices and Van den Bergh's tests indicated that a liver dysfunction occurred during infection. According to the "direct reaction" obtained by the Van den Bergh test an obstructive jaundice was present. Under these conditions the excretion of bilirubin is blocked due to the obstruction of the biliary passages.

According to Watson (1946) the bilirubin-globin complex is made from hemoglobin in the reticulo-endothelial system. Since hemoglobin is thus implicated, complete blood counts were performed. The results of these determinations demonstrated that there was a slight decrease of hemoglobin in the infected rats, evidenced by a higher color index as compared to that of normal animals.

Also of importance is the observation that rats recovered from infection exhibited a pronounced increase in leucocyte count as compared to normal animals and animals having an acute infection. It seems that the relatively high leucocyte count is definitely associated with the high degree immunity to tularemia exhibited by recovered rats. Further investigation of this phenomenon should be con-

ducted although the scope of this project does not include immunological studies.

The results of the experiments on the effect of administering amino acids singly and in a mixture on the course of infection, reveal that the average day of death was approximately the same in all groups. It may be seen, however, that the total number of animals dead in the group of rats receiving the amino acid mixture and approximately $3.5 \times 10^3$ organisms/ml. was slightly higher than the corresponding group receiving only physiological saline. This may indicate that the fortification of the host with amino acids may provide the organism in the host with a readily available supply of nutrients. If true, this conceivably could enhance their virulence by either selecting more virulent forms, in which case the amino acids would be acting as selective agents, or the amino acids enabled the organism to metabolize much more rapidly thus becoming much more destructive. Attempts to detect colonial variants of Bacterium tularense isolated from the infected animals were unsuccessful, although this does not eliminate the possibility of selection of a more virulent population.

The results of the effect of various diets on the course of infection shows that the average day of death is not altered significantly regardless of diet. From this it appears that the state of nitrogen balance of the host may not be of primary importance as far as the disease is concerned.

The data reported in this paper have not enabled us to determine the fate of the free amino acids of the blood of tularemic rats.

Nevertheless, valuable information has been obtained concerning metabolic changes during infection. For the first time a systematic study has been made of the clinical biochemistry and hematology of tularemia in either animals or humans. The data obtained will serve as a basis for similar studies on other animals and also for continued research on tularemia in the white rat.

## REFERENCES


Folin, Otto and Wu, Hsien, 1919 A system of blood analysis J. Biol. Chem., 38:81-110.

Folin, Otto and Wu, Hsien, 1920 A system of blood analysis. Supplement I. A simplified and improved method for determination of sugar. J. Biol. Chem. 41:367-374.

Greenberg, David M., 1924 The colorimetric determination of the serum proteins. J. Biol. Chem., 82:545-550.

Hawk, Philip B., Oser, Bernard L. and Summerson, Wm. H., 1948 Practical Physiological Chemistry. 12th Edition The Blakiston Company, Philadelphia, Pa.

Hepler, Opal E., 1951 Manual of Clinical Laboratory Methods. 4th Edition. Charles C. Thomas, Publisher, Springfield, Illinois.

Karr J. Lab. Clin. Med. 9 3(1924)

Karr, W. G. and Clark, J. H. 1941 Comparison of various hemoglobin methods as performed in hospital and physicians' laboratories. Am. J. Chem. Path., Tech. Suppl. 5:127-147.

Pullen, Roscoe L. and Stuart, Byron M., 1945 Tularemia analysis of 225 cases. J. Amer. Med. Assoc., 129:495-500.

Van den Bergh, A. A. H., 1921 Diazo test for bilirubin in blood. Presse med., 29:441. (cited Practical Physiological Chemistry. 12th Edition, The Blakiston Company, Philadelphia, Pa.)

Watson, C. J., 1946 Some newer concepts of the natural derivatives of hemoglobin. Blood, 1:99

Table 1

STATISTICAL EVALUATION OF THE NON PROTEIN NITROGEN DETERMINATIONS FOR NORMAL AND RECOVERED RATS

| Number of Animals | Condition of Animal | NPN mgm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 38 | Normal | 13.20 | 3.12 | 1.76 | 12.72-13.68 | 2.50±0.73 |
| 37 | Infected | 10.70 | 3.96 | 1.99 | 10.15-11.25 | |

Computations:

$$\text{Variance} = \frac{S(X - \bar{X})^2}{N - 1}$$

$$\text{Standard Deviation} = \sqrt{\frac{S(X - \bar{X})^2}{N - 1}}$$

$$\text{90 per cent confidence Interval for mean} = \bar{X} \pm t_{90} \frac{S}{\sqrt{n}}$$

$$\text{90 per cent confidence interval of the difference of the means} = \bar{X} - \bar{X}' \pm z_{90} \sqrt{\frac{S^2}{N} + \frac{S'^2}{N'}}$$

Table 2

STATISTICAL EVALUATION OF THE BLOOD UREA NITROGEN DETERMINATIONS FOR NORMAL AND INFECTED RATS*

| Number of Animals | Condition of Animal | BUN Mean mgm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean mgm per cent | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 46 | Normal | 11.30 | 7.90 | 2.82 | 10.59-12.01 | 1.10 ± 0.86 |
| 33 | Infected | 10.10 | 3.34 | 1.82 | 9.56-10.64 | |

* Computations same as on Table 1.

Table 3

STATISTICAL EVALUATION OF THE URINE UREA NITROGEN DETERMINATIONS FOR NORMAL AND INFECTED RATS*

| Number of Animals | Condition of Animal | UUN Mean mgm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 27 | Normal | 11.60 | 8.10 | 2.84 | 10.66-12.54 | 0.60±1.35 |
| 30 | Infected | 12.20 | 11.8 | 3.43 | 11.14-13.26 | |

*Computations same as Table 1.

Table 4

STATISTICAL EVALUATION OF THE SERUM PROTEIN DETERMINATIONS FOR NORMAL AND INFECTED RATS*

| Number of Animals | Condition of Animal | Total Protein Mean gm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the mean |
|---|---|---|---|---|---|---|
| 10 | Normal | 5.90 | 0.57 | 0.25 | 5.76-6.04 | 0.60±0.14 |
| 10 | Infected | 5.30 | 0.017 | 0.13 | 5.22-5.38 | |

*Computations same as on Table 1.

Table 5

STATISTICAL EVALUATION OF THE BLOOD GLUCOSE DETERMINATIONS FOR NORMAL AND INFECTED RATS*

| Number of Animals | Condition of Animal | Blood Glucose Mean mgm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 28 | Normal | 108 | 48.60 | 6.90 | 105.79-110.21 | 17.00-3.16 |
| 40 | Infected | 91 | 106 | 10.30 | 88.26-93.74 | |

*Computations same as on Table 1.

Table 6

STATISTICAL EVALUATION OF THE ICTERIC INDICES FOR NORMAL AND INFECTED RATS*

| Number of Animals | Condition of Animal | Icteric index Mean | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 45 | Normal | 6.50 | 3.90 | 1.98 | 6.00-7.00 | 6.80-240 |
| 34 | Infected | 16.30 | 69.1 | 8.34 | 13.99-18.71 | |

*Computations same as on Table 1.

Table 7

RESULTS OF VAN DEN BERGH DETERMINATIONS FOR BILIRUBIN IN NORMAL AND INFECTED RATS

| Number of Animals | Condition of Animal | Result |
|---|---|---|
| 34 | Normal | Negative |
| 34 | Infected | 15 direct reaction<br>19 Negative |

Table 8

COMPLETE BLOOD COUNTS OF NORMAL AND INFECTED RATS

| Number of Animals | Condition of Animal | Red Blood Cell Count | | White Blood Cell Count | | Hemoglobin | |
|---|---|---|---|---|---|---|---|
| | | Mean | Range | Mean | Range | Mean | Range |
| 10 | Normal | 6,64[illegible],000 | 5,450,000 to 8,210,000 | 6,730 | 4,500 to 9,200 | 84.6% | 79.5 to 88% |
| 20 | Infected | 6,870,000 | 5,800,000 to 8,100,000 | 8,360 | 6,200 to 10,300 | 82.2% | 79.5 to 86% |

| Number of Animals | Condition of Animals | Color Index | | Differential Segmented Neutrophiles | | Lymphocytes | |
|---|---|---|---|---|---|---|---|
| | | Mean | Range | Mean | Range | Mean | Range |
| 10 | Normal | 1.56 | 1.26 - 1.96 | 33 | 32-40 | 66 | 60-70 |
| 10 | Infected | | 1.39 - 1.98 | 34 | 28-40 | 65 | 60-71 |

Table 9

STATISTICAL EVALUATION OF THE BLOOD NON PROTEIN NITROGEN FOR RECOVERED AND REINFECTED RATS*

| Number of Animals | Condition of Animals | NPN Mean mgm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 38 | Normal | 13.20 | | | | |
| 10 | Recovered | 12.90 | 0.75 | 0.87 | 12.40-13.40 | 0.20-0.62 |
| 10 | Reinfected | 13.10 | 0.68 | 0.83 | 12.52-13.58 | |

*Computations same as on Table 1.

Table 10

STATISTICAL EVALUATION OF THE BLOOD UREA NITROGEN FOR RECOVERED AND REINFECTED RATS*

| Number of Animals | Condition of Animals | BUN Mean mgm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 46 | Normal | 11.30 | | | | |
| 10 | Recovered | 11.50 | 0.865 | 0.930 | 10.96-12.04 | 0.1-0.62 |
| 10 | Reinfected | 11.60 | 0.600 | 0.780 | 11.14-12.06 | |

*Computations same as on Table 1.

Table 11

STATISTICAL EVALUATION OF THE SERUM PROTEIN DETERMINATIONS FOR RECOVERED AND REINFECTED RATS*

| Number of Animals | Condition of Animals | Mean gm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 10 | Normal | 5.90 | | | | |
| 10 | Recovered | 5.80 | 0.061 | 2.245 | 5.66-5.94 | 0.10-1.63 |
| 10 | Reinfected | 5.90 | 0.038 | 0.197 | 5.78-6.02 | |

*Computations same as on Table 1.

Table 12

STATISTICAL EVALUATION OF THE ICTERIC INDICES FOR RECOVERED AND REINFECTED RATS*

| Number of Animals | Condition of Animals | Mean mgm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 45 | Normal | 6.50 | | | | |
| 10 | Recovered | 6.60 | 0.62 | 0.73 | 6.18-7.02 | 0.10±0.49 |
| 10 | Reinfected | 6.50 | 0.27 | 0.53 | 6.18-6.82 | |

*Computations same as on Table 1.

Table 13

RESULTS OF VAN DEN BERGH DETERMINATIONS FOR BILIRUBIN OF RECOVERED AND REINFECTED RATS

| Number of Animals | Condition of Animal | Result |
|---|---|---|
| 10 | Recovered | Negative |
| 10 | Reinfected | Negative |

Table 14

STATISTICAL EVALUATION OF THE BLOOD SUGAR DETERMINATIONS FOR RECOVERED AND REINFECTED RATS*

| Number of Animals | Condition of Animals | Mean mgm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the means |
|---|---|---|---|---|---|---|
| 28 | Normal | 108 | | | | |
| 10 | Recovered | 109.04 | 59.60 | 7.72 | 104.59-113.41 | $4 \pm 4.75$ |
| 10 | Reinfected | 105.03 | 22.50 | 4.75 | 102.34-107.66 | |

*Computations same as on Table 1.

Table 15

STATISTICAL EVALUATION OF THE URINE UREA NITROGEN DETERMINATIONS FOR RECOVERED AND REINFECTED RATS*

| Number of Animals | Condition of Animals | UUN Mean mgm per cent | Variance | Standard Deviation | 90 per cent confidence interval for mean | 90 per cent confidence interval of the difference of the mean |
|---|---|---|---|---|---|---|
| 27 | Normal | 11.60 | | | | |
| 12 | Recovered | 11.71 | 0.25 | 0.50 | 11.44-12.00 | 0.50±0.51 |
| 12 | Reinfected | 12.20 | 0.57 | 0.76 | 12.00-12.70 | |

*Computations same as on Table 1.

Table 16

COMPLETE BLOOD COUNTS ON RECOVERED AND REINFECTED RATS*

| Number of Animals | Condition of Animals | Red Blood Cell Count | | White Blood Cell Count | | Hemoglobin | |
|---|---|---|---|---|---|---|---|
| | | Mean | Range | Mean | Range | Mean | Range |
| 10 | Normal | 6,641,000 | 5,450,000 to 8,210,000 | 6,730 | 4500 to 9200 | 84.6% | 79.5 to 88% |
| 10 | Recovered | 6,029,000 | 5,070,000 to 7,280,000 | 11,130 | 9,800 to 13,500 | 85.4% | 76 to 110.5% |
| 10 | Reinfected | 6,583,000 | 5,900,000 to 7,220,000 | 11,109 | 8,900 to 13,200 | 86.5% | 77 to 97% |

| Number of Animals | Condition of Animals | Color Index | | Differential | | | |
|---|---|---|---|---|---|---|---|
| | | | | Segmented neutrophiles | | Lymphocytes | |
| | | Mean | Range | Mean | Range | Mean | Range |
| 10 | Normal | 1.56 | 1.26 to 1.96 | 33% | 32 to 40% | 66% | 60-71% |
| 10 | Recovered | 1.43 | 1.06 to 1.89 | 35% | 29 to 41% | 64% | 58-70% |
| 10 | Reinfected | 1.53 | 1.37 to 1.73 | 36% | 30 to 41% | 63% | 59-67% |

*Computations same as in Table 1.

Table 17

EFFECT OF ADMINISTERING AMINO ACIDS ON THE COURSE OF INFECTION

| Infecting Dose | Additional Treatment | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 15 | ADD[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $3.5X10^{4}$* | | 0[a] | 0 | 31 | 41 | 46 | 49 | 49 | 50 | | | | | 50 | 3.68 |
| | | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | | | | | 50 | |
| $3.5X10^{4}$ | Amino | 0 | 0 | 19 | 32 | 36 | 37 | 38 | 38 | 40 | | | | 40 | 4.00 |
| | Acid | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | 40 | | | | 40 | |
| $3.5X10^{4}$ | mixture | 0 | 0 | 15 | 27 | 29 | 29 | 30 | | | | | | 30 | 3.68 |
| | P/ss | 30 | 30 | 30 | 30 | 30 | 30 | 30 | | | | | | 30 | |
| $3.5X10^{3}$ | -- | 0 | 0 | 10 | 28 | 36 | 42 | 52 | 54 | | | | | 54 | 4.90 |
| | | 90 | 90 | 90 | 90 | 90 | 90 | 90 | 90 | | | | | 90 | |
| $3.5X10^{3}$ | Amino | 0 | 1 | 17 | 46 | 56 | 70 | 78 | 79 | 81 | 84 | | | 84 | 4.90 |
| | acid | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | 125 | | | 125 | |
| $3.5X10^{3}$ | mix | 0 | 0 | 1 | 19 | 25 | 31 | 37 | 37 | 37 | 39 | 40 | | 40 | 5.60 |
| | P/ss | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 | 75 | | 75 | |
| $3.5X10^{2}$ | -- | 0 | 0 | 0 | 0 | 9 | 12 | 15 | 16 | 17 | 19 | | | 19 | 6.35 |
| | | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | 36 | | | 36 | |
| $3.5X10^{2}$ | Amino | 0 | 0 | 0 | 6 | 20 | 24 | 29 | 31 | 31 | 32 | 35 | | 35 | 6.05 |
| | acid | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | 65 | | 65 | |
| $3.5X10^{2}$ | mix | 0 | 0 | 0 | 4 | 6 | 10 | 11 | 14 | 15 | | | | 15 | 6.00 |
| | P/ss | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | 19 | | | | 19 | |
| $3.5X10^{1}$ | -- | 0 | 0 | 0 | 0 | 5 | 7 | 8 | 9 | 9 | | | | 9 | 5.80 |
| | | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | | | | 20 | |
| $3.5X10^{1}$ | Amino | 0 | 0 | 0 | 2 | 3 | 5 | 6 | 6 | 9 | 9 | 10 | 10 | 11 | 5.95 |
| | Acid mix | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | |
| $3.5X10^{1}$ | P/ss | 0 | 0 | 0 | 4 | 5 | 10 | 11 | 12 | 12 | 13 | 14 | | 14 | 6.20 |
| | | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | | 25 | |
| 3.5 | -- | 0 | | | | | | | | | | | | 0 | 0.00 |
| | | 25 | | | | | | | | | | | | 25 | |
| 3.5 | Amino | 0 | | | | | | | | | | | | 0 | 0.00 |
| | Acid mix | 15 | | | | | | | | | | | | 15 | |
| 3.5 | P/ss | 0 | | | | | | | | | | | | 0 | 0.00 |
| | | 15 | | | | | | | | | | | | 15 | |
| -- | Cystine | 0 | | | | | | | | | | | | 0 | 0.00 |
| | | 15 | | | | | | | | | | | | 15 | |
| -- | Arginine | 0 | | | | | | | | | | | | 0 | 0.00 |
| | | 15 | | | | | | | | | | | | 15 | |
| -- | Amino | 0 | | | | | | | | | | | | 0 | 0.00 |
| | Acid mix | 20 | | | | | | | | | | | | 20 | |
| -- | P/ss | 0 | | | | | | | | | | | | 0 | 0.00 |
| | | 10 | | | | | | | | | | | | 10 | |

a) Mortality ratio= $\frac{\text{Dead}}{\text{Total Tested}}$ b) ADD= Average day of death

c) P/ss = Physiological saline solution

*Concentration of organisms.

Table 18

EFFECT OF VARIOUS DIET ON THE COURSE OF INFECTION

| Infecting Dose CE | Per cent Protein | Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | ADD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $3.5X10^3$ | 5% | 0/10 | 0/10 | 6/10 | 8/10 | 8/10 | | | | | | | | | | 8/10 | 3.24 |
| $3.5X10^3$ | 20% | 0/10 | 1/10 | 2/10 | 4/10 | 5/10 | 7/10 | | | | | | 8/10 | | | 8/10 | 4.30 |
| $3.5X10^3$ | 30% | 0/10 | 0/10 | 3/10 | 6/10 | 7/10 | | | | | | | | | | 7/10 | 3.70 |
| $3.5X10^3$ | Normal | 0/10 | 0/10 | 2/10 | 3/10 | 4/10 | 6/10 | | | | | | | | | 6/10 | 3.86 |
| $3.5X10^2$ | 5% | 0/9 | 0/9 | 4/9 | 5/9 | 6/9 | | | | | | | 7/9 | | | 7/9 | 4.70 |
| $3.5X10^2$ | 20% | 0/9 | 0/9 | 0/9 | 2/9 | 4/9 | 4/9 | 5/9 | | 6/9 | | | | | | 6/9 | 5.65 |
| $3.5X10^2$ | 30% | 0/10 | 0/10 | 0/10 | 2/10 | 5/10 | 6/10 | | 7/10 | | | | | | | 7/10 | 5.30 |
| $3.5X10^2$ | Normal | 0/8 | 0/8 | 2/8 | 3/8 | 3/8 | 4/10 | | | | | | | | | 4/10 | 4.00 |