after administration of S^{35} taurine probably represents normal metabolites of taurine such as taurocyamine and carbamyl taurine(13, 14). The identity of these products is of interest but, since they are found in the urine of fasting individuals in relatively large amounts, they probably reflect the low level of body taurine rather than the synthesis of unique metabolites. The reason for the increased excretion of taurine metabolites in fasting states remains unknown.

Summary. The metabolism of S^{35} taurine has been studied in normal individuals and mongols. The latter contains a group that excretes very low amounts of urinary taurine and urine from these individuals yields radiograms similar to those obtained from fasting normal individuals. These radiograms are characterized by the appearance of taurine metabolites in relatively greater amounts than taurine itself. In contrast, normal individuals after a meal produce patterns characterized by large taurine peaks compared to taurine metabolites. When administered loads of taurine, the low excretors among the mongols excrete much less of the load than do higher excretors. The low excretors behave as if deficient in body taurine.

1. Goodman, H. O., King, J. S., Jr., Thomas, J. J., Nature, 1964, v204, 650.

2. King, J. S., Goodman, H. O., Thomas, J. J., Acta Genet. Stat. Med., accepted for publication.

3. King, J. S., Wainer, A., Goodman, H. O., Thomas, J. J., Proc. of 1965 Technicon Symposium, Mediad, to be published.

4. Gordon, C. F., Wolfe, A. L., Anal. Chem., 1960, v32, 574.

5. Wainer, A., Biochem. Biophys. Res. Commun., 1964, v16, 141.

6. Turner, F. P., Brum, V. C., J. Surg. Res., 1960, v4, 423.

7. Pentz, E. I., Moss, W. T., Denko, C. W., J. Clin. Endocrin. and Metab., 1959, v19, 1126.

8. Gilbert, J. B., Ku, Y., Rogers, L. L., Williams, R. J., J. Biol. Chem., 1960, v235, 1055.

9. Agur, A., Pinsky, A., Israel J. Chem., 1964, v2, 318.

10. Chatagner, F., Tabechian, H., Bergeret, B., Biochem. and Biophys. Acta, 1954, v13, 313.

11. Hope, D. D., J. Neurochem., 1957, v1, 364.

12. Swan, P., Wentworth, J., Linkswiler, H., J. Nutrition, 1964, v84, 220.

13. Thoai, N.-V., Roche, J., Olomuchi, A., Biochim. Biophys. Acta, 1954, v14, 448.

14. Schram, E., Crokaert, R., Biochem. J., 1957, v66, 20.

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Experimental Glomerulonephritis. IX. Factors Influencing the Development of Kidney in Adjuvant Nephritis in Rats.*† (30741)

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The production of a nephritis in rats by immunization with homologous kidney in adjuvant was first reported in 1959(1). After 6-8 intraperitoneal injections of homologous kidney in complete Freund's adjuvant, rats developed a disease very similar to the human nephrotic syndrome with hyperlipemia, reversal of albumin-globulin ratios and massive proteinuria. In spite of considerable study (2-6), the etiologic and pathogenetic mechanisms operative in this disease have still not been defined; and therefore, the name "kidney in adjuvant nephritis" rather than "autoimmune nephritis" with its pathogenetic implications has been used here.

The experiments to be reported were undertaken in an attempt to define more clearly some of the factors important in the induction of this disease. *First,* the immunization procedures were altered by increasing the frequency of injections and by altering the components of the adjuvant. *Second,* the role of the host animal was assessed by immuniz-

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ing four different strains of rats with homologous kidney in complete Freund's adjuvant and comparing the subsequent severity of the nephritis in each strain. *Third,* the characteristics of the renal antigen were also studied in several ways. The efficacies of isologous, homologous and heterologous kidney antigens in producing the nephritis were compared. In addition, the antigen was altered by chemical means and the amount of antigen used in a course of immunization was varied. It was found that the nature of the disease could be modified by manipulating any of these 3 components.

Materials and methods. Rats: Three inbred, isologous strain (Lewis, Fisher, Buffalo) and one random bred strain (Sprague-Dawley) were used as test animals. Lewis rats were obtained from Microbiological Associates, Walkersville, Md.; Fisher and Buffalo rats from Simonsen Laboratories, Gilroy, Calif.; and Sprague-Dawley rats from Holtzman Rat Co., Madison, Wisc. Only male rats were used. They weighed between 150 and 250 grams at start of experiments.

Adjuvant: Freund's complete adjuvant was usually made by mixing 9 parts of Bayol F with 1 part of Arlacel C, and adding 2.0 mg of *Mycobacterium tuberculosis* (Strain H37 Ra-Difco Laboratories) per ml. In one experiment, Arlacel A was substituted for Arlacel C. In another experiment, *Mycobacterium tuberculosis* was replaced by merthiolate-killed nephritogenic streptococci.

Streptococci: Group A, beta-hemolytic streptococci, type 12, strain 620-0 obtained through Dr. Elayne L. Updyke, Communicable Disease Center, Chamblee, Ga., were grown on blood agar and transferred every 24 hours. Prior to use, they were subcultured on Eugon broth for 18 hours at 37°C and then killed by 24 hours in a 0.01% merthiolate solution. When substituted for *Mycobacterium tuberculosis,* one ml of suspension containing 1×10^8 micro-organisms was combined with one ml of adjuvant.

Renal antigen: Normal Lewis, Fisher, Buffalo and Sprague-Dawley unperfused rat kidneys were used, as either isologous or homologous antigens. After homogenization in a Waring blendor with isotonic saline (1:1, V/V), they were centrifuged at $1600 \times g$ for 90 minutes at 4°C. The sediment was then discarded and the supernatant was stored at −20°C. The protein concentration of the different supernatants varied between 45 and 65 mg/ml. For heterologous antigen, homogenized normal human kidney in saline, in proportions of 1:2 (V/V), was used. The protein concentration of this preparation was 25 mg/ml.

Immunization: In all experiments, 0.4 ml of kidney preparation was emulsified in 1.0 ml of complete Freund's adjuvant. When streptococci were used instead of *Mycobacterium tuberculosis,* the mixture employed consisted of 0.5 ml of adjuvant, 0.5 ml of streptococcal suspension, and 0.4 ml of kidney preparation. Individual injections were 0.25 ml of the respective mixtures and were always given intraperitoneally. For isologous kidney studies, rats received 10 or 11 bimonthly injections, *i.e.,* every two weeks, the equivalent of approximately 45 mg of kidney protein. With homologous kidney, 2 different immunization schedules were used, either 6-10 bimonthly injections, immunization being stopped when proteinuria exceeded 150 mg per 24 hours, or 8 weekly injections, irrespective of proteinuria. All homologous kidney recipients received between 25 and 45 mg of protein. Thirteen heterologous kidney injections totaling approximately 25 mg of protein were given to each recipient.

Alterations in amount of kidney antigen: In one experiment, Sprague-Dawley kidney homogenate was diluted 1:3 prior to incorporation in adjuvant. A group of Sprague-Dawley rats was then given 10 bimonthly injections of this diluted antigen in adjuvant, the equivalent of 15 mg of protein. In a second experiment, Lewis rats received 8 weekly injections of Sprague-Dawley kidney diluted 1:5, or a total of 6.4 mg protein. Another group of Lewis rats received a single 0.5 ml injection of undiluted Sprague-Dawley kidney in complete adjuvant containing approximately 9.2 mg of protein.

Chemical alteration of kidney antigen: Sprague-Dawley kidney supernatant that was to be chemically altered was first prepared as described above. The proteins were then

coupled to diazonium derivatives of both arsanilic and sulfanilic acids by a modification of the method described by Baker *et al*(7). The diazonium derivatives were prepared by dissolving (separately) 0.069 g of sulfanilic acid and 0.078 g of arsanilic acid in a mixture of 0.9 ml of 1 N HCl and 0.72 ml of 0.5 N $NaNO_2$. The solutions were brought to 5 ml with distilled H_2O and 2.5 ml of each preparation added dropwise at 0°C with constant stirring, to 560 mg of protein in 20 ml of phosphate buffer, pH 7.5 and molarity 0.1. The pH was maintained at 7.5 to 7.8 by addition of 0.1 N NaOH. The final pH was adjusted to 7.8 and the conjugate stored overnight at 4°C. The free, non-bound derivatives were removed by exhaustive dialysis against 0.15 M NaCl at 4°C. The number of azo groups per unit weight of protein was not determined; however, 1.8 moles of each derivative were offered. Two groups of rats received 8 weekly injections of this altered kidney in complete Freund's adjuvant. The rats in the first group each received a total of 32 mg of protein; those in the second received 6.4 mg.

Proteinuria in rats was measured by precipitation with 3% sulfosalicylic acid. Normals of the 4 strains of rats used within the weight range employed had proteinurias averaging approximately 6 mg per 24 hours.

Results. 1. *Effects of manipulation of immunization procedures.* a) Forty Lewis rats were injected weekly in an attempt to see if the induction period of the disease could be shortened. The average proteinuria of these animals was compared to the proteinuria present in 40 Lewis rats that received bimonthly injections (Table I). It was found that weekly injections significantly hastened the development of nephritis. Abnormal proteinuria appeared only one week earlier with this immunization schedule; however, it then increased much more rapidly than it did with bimonthly injections. Average proteinuria in excess of 200 mg a day was seen by the eleventh week after the start of weekly injections, while comparable values were not obtained until the 16th to 18th week after the start of bimonthly immunization (Table II).

TABLE I. Comparison of Weekly and Bimonthly Injections of Homologous Kidney in Complete Freund's Adjuvant in Production of Nephrotic Syndrome in Lewis Rats.

Frequency of injections	Avg proteinuria (mg/24 hr) Weeks after first injection 6	7	8	9	10	11
Weekly	10	23	62	114	120	203
Bimonthly	6	9	22	19	60	66

TABLE II. Susceptibility of 4 Different Strains of Rats to the Induction of Nephrotic Syndrome by Bimonthly Immunization with Sprague-Dawley Kidney in Adjuvant.

Strain of rat	Avg proteinuria (mg/24 hr) Weeks after first injection 8	10	12	14	16	18	20
Lewis	22	60	111	180	187	249	—
Fisher	5	16	55	122	158	—	155
Buffalo	7	6	7	10	7	—	10
S.D.	9	13	30	34	69	133	169
S.D.*	17	35	—	76	134	227	299

* Arlacel A used in place of Arlacel C.

b) An attempt was made to see if nephritis could be produced if *Mycobacterium tuberculosis* was replaced by another micro-organism. Ten Lewis rats received 8 weekly injections of Sprague-Dawley kidney emulsified in incomplete adjuvant and killed nephritogenic streptococci. Abnormal proteinuria was not seen in any of these rats, although checked for regularly from the second week on. In addition, renal tissue taken at sacrifice was negative when examined by fluorescence microscopy for the presence of rat gamma globulin, rat complement or streptococcal antigens, and was normal histologically.

c) In one experiment Arlacel C was replaced by the more phlogogenic Arlacel A. Ten Sprague-Dawley rats were then injected bimonthly with Sprague-Dawley kidney in this adjuvant. The proteinuria obtained in these animals is compared in Table II with the proteinuria seen in Sprague-Dawley rats after immunization with Arlacel C adjuvant. When Arlacel A was used, abnormal proteinuria developed somewhat earlier. More striking, however, was the increment in the rate at which proteinuria increased and the eventual levels attained. From the 8th week on, proteinuria in the Arlacel A treated rats

was approximately twice as great as that seen in the group treated with Arlacel C. In addition, every rat in the Arlacel A group had abnormal proteinuria, in contrast to 75% of rats with abnormal proteinuria in the Arlacel C group. Morphologic examination of the glomeruli of these rats showed, as expected, a more severe lesion without, however, qualitative morphologic differences between the two groups.

2. *Influence of host on production of nephrotic syndrome.* Groups of 20 Fisher, Buffalo and Sprague-Dawley rats, and 40 Lewis rats, were immunized with homologous kidney in complete Freund's adjuvant. Sprague-Dawley kidney was used as the homologous antigen for all 4 strains. Injections were given bimonthly and were continued until either proteinuria exceeded 150 mg a day or a rat had received 10 injections. Very marked strain variations in susceptibility to induction of disease were found (Table II). Lewis rats developed nephropathy most readily, with abnormal proteinuria occurring by the 8th week after the start of injections, and increasing progressively thereafter. The incidence of disease in this strain was approximately 95%. Fisher rats were the second most susceptible of the strains tested. Ninety per cent of immunized Fisher rats developed proteinuria greater than 100 mg a day. However, abnormal protein excretion developed later and did not become as heavy as that seen in Lewis rats. Morphologically the disease in Fisher rats was identical to that present in Lewis rats. Nephritis could not be produced in the Buffalo strain. All of the Buffalo rats received a full course of 10 injections but in no case was abnormal proteinuria seen. In addition, host gamma globulin and complement were not present in the kidneys examined by fluorescence, and morphologic examination revealed no abnormalities. Of the 3 strains of rats found susceptible to this disease, the random-bred Sprague-Dawley animals, in which the disease was originally produced(1) had the least proteinuria. Significantly elevated levels of proteinuria were not seen in these animals until 16 weeks after the start of immunization, in contrast to Lewis and Fisher rats where comparable levels had been reached at the 10th and 12th week respectively. The per cent of Sprague-Dawley rats with proteinuria (75%) was also lower than the 90%± incidence of disease in the 2 inbred strains.

TABLE III. Effectiveness of Isologous Kidney in Complete Adjuvant in Production of Nephrotic Syndrome in 3 Inbred Strains of Rats.*

Strain of rat	Avg proteinuria (mg/24 hr) Weeks after first injection 8	10	12	14	16	18	20	25	27
Lewis	5	8	9	8	8	10	15	50	36
Fisher	1	3	5	3	3	6	4	14	—
Buffalo	3	5	6	5	10	16	13	13	—

* 10 Bimonthly intraperitoneal injections of kidney in adjuvant.

3. *Efficacy of isologous, homologous and heterologous kidney antigens in production of nephrotic syndrome.* a) Attempts were made to induce nephritis in all 3 inbred strains by immunizing groups of 15-30 rats with isologous kidney in adjuvant (Table III). All animals in these experiments were injected bimonthly and all received a full course of 10 injections. In Lewis rats, the strain found most susceptible to homologous kidney in adjuvant, abnormal proteinuria was first seen in individual animals by the 19th week but the group average was not significantly elevated until the 25th week after the start of immunization. Subsequent protein checks, not listed in the table, were done on some of the rats and revealed that a moderately increased proteinuria persisted. Fourteen per cent of the 22 Lewis rats that survived through the period of observation had proteinuria in excess of 100 mg a day and an additional 14% had a proteinuria of between 35 and 100 mg a day. Moreover, the alterations seen in the kidneys of these rats were morphologically identical to those present after homologous kidney in adjuvant. Isologous kidney in adjuvant did not, however, produce clinical disease in Fisher or Buffalo rats. In these 2 strains, the levels of proteinuria were higher in the later weeks of observation than in the earlier weeks but this was due, in part, to the increasing size of the rats and also was possibly a manifes-

TABLE IV. Comparative Efficacy of Lewis and Sprague-Dawley Kidney as Homologous Antigens.

Type of immunization*	Avg proteinuria (mg/24 hr) — Weeks after first injection — 8	10	12	14	16	18	20
S.D. into Fisher	5	16	55	122	158	—	155
Lewis into Fisher	14	—	30	67	50	109	—
S.D. into Buffalo	7	6	7	10	7	—	10
Lewis into Buffalo	8	—	5	7	4	7	7
S.D. into S.D.	9	13	30	34	69	133	169
Lewis into S.D.	20	13	28	—	37	—	—

* Immunization consisted of 10 bimonthly injections for all but Sprague-Dawley rats receiving Lewis kidney, where 8 weekly injections were given.

tation of the nephrotoxic action of the Freund's adjuvant itself(8). No individual rats in either strain had proteinuria in excess of 35 mg a day and morphologic examination of the kidneys of immunized Buffalo rats revealed no abnormalities. Fisher rat kidneys, however, did show occasional focal thickening of the glomerular basement membrane and by immunofluorescence host gamma globulin and complement were present in glomeruli in a light granular pattern.

b) Homologous kidney: the effectiveness of homologous kidney in adjuvant immunization was shown in Table II. Further homologous kidney studies were done in the Fisher, Buffalo and Sprague-Dawley strains using Lewis kidney as the homologous antigen. Ten Fisher and 10 Buffalo rats received 10 bimonthly injections of Lewis kidney in adjuvant, while the groups of 10 Sprague-Dawley rats received 8 weekly injections. Table IV shows the proteinuria obtained in the 3 strains using Lewis kidney. For ease of comparison, the proteinurias seen after immunization with Sprague-Dawley kidney in complete adjuvant are also included in the Table. Lewis kidney was a less effective antigen than Sprague-Dawley kidney. In Fisher rats, the group average eventually exceeded 100 mg of proteinuria a day but this occurred 4 weeks later than it did with Sprague-Dawley kidney in adjuvant. In addition, only 50% of the Fisher rats immunized with Lewis kidney in adjuvant had a proteinuria in excess of 100 mg a day. In Sprague-Dawley rats, Lewis kidney in adjuvant produced only a mild proteinuria by the 16th week, in striking contrast to Sprague-Dawley kidney. This difference was particularly significant, since the Sprague-Dawley rats receiving Lewis kidney were immunized by weekly injections rather than bimonthly injections. Buffalo rats, as was expected, did not develop any proteinuria with Lewis kidney.

c) Heterologous kidney: Lewis rats were the only rats immunized with human kidney. This proved to be a less effective antigen than homologous kidney, however, it was more efficient than isologous kidney. A group of 15 Lewis rats received 13 bimonthly injections of human kidney in complete adjuvant. Abnormal proteinuria developed in individual rats during the 14th week but the group average did not become significantly elevated until the 19th week. Moreover, the group average never exceeded 100 mg of protein a day, being between 80 and 90 mg from the 28th week on. While only 25 mg of human kidney was used for immunization in contrast to a range of 25-45 mg of homologous kidney, it does not seem likely that this quantitative difference could account for the difference in severity of the disease. Morphologically the disease produced by immunization with heterologous kidney was identical to that produced by homologous kidney.

4. *Effect of altering the renal antigen.* The amount of kidney antigen given during a course of immunization was decreased to see if the resultant nephritis would be less severe. Sprague-Dawley kidney was used as the antigen in each of these experiments. One group of Lewis rats received 8 weekly injections of kidney in adjuvant after the antigen had been diluted 5 times to a concentration of 11 mg of protein per ml prior to incorporation in adjuvant. These rats therefore received a total of 6.4 mg of protein. Proteinuria in these rats developed somewhat later than it had in Lewis rats that received 32 mg of undiluted kidney antigen in the same immunization schedule, but levels were comparable by the eleventh week after the start of immunization (Table V). In the rats that received a total of only 6.4 mg of protein, however, the nephritis as judged by proteinuria, tended to improve thereafter. Another group of Lewis

TABLE V. Efficiency of Native and Altered Renal Antigens in Inducing Nephrotic Syndrome.

Antigen	Rats	Injections	Days between injections	Mg prot. per injection	Avg proteinuria (mg/24 hr) Weeks after first injection 7	9	11	12	14	16	18	20	22
S.D.	40 L	6–10	7	4.5	23	114	203	—	216				
S.D.	20 L	8	7	.8	14	61	195	—	148				
S.D.	5 L	1	—	9.2	6	—	17	—	15	30	21	—	52
S.D.	20 SD	6–10	14	4.5	10	14	16	30	34	69	133	169	—
S.D.	10 SD	10	14	1.5	18	18	—	52	85	—	97	75	—
AS-SD	10 L	8	7	4	57	273	352						
AS-SD	10 L	8	7	.8	47	263	203						

L—Lewis; SD—Sprague-Dawley; AS-SD—Arsanil-sulfanil labeled Sprague-Dawley kidney.

rats received a single injection of 0.5 ml of kidney in adjuvant, containing 9.2 mg of kidney protein. Abnormal proteinuria was not seen in these rats until 16 weeks after the injection and the levels attained were never more than moderate. In another experiment, Sprague-Dawley rats were immunized with kidney antigen that had been diluted 1:3. These animals each received 10 bimonthly injections, the equivalent of 15 mg of protein. Elevated levels of proteinuria developed earlier in this group than in Sprague-Dawley rats receiving the full amounts of homologous kidney but, as with Lewis rats, the nephritis produced by diluted antigen tended to diminish after the cessation of injections (Table V).

Two groups of Lewis rats were immunized with Sprague-Dawley kidney that had been coupled to arsanilic and sulfanilic acid derivatives. For one group the altered antigen was used at full concentration of 56 mg of protein/ml. For the second group, the kidney preparation was diluted to a concentration of 11 mg of protein/ml. Both groups were given 8 weekly injections so that the first group received a total of 32 mg of protein and the second group 6.4 mg. It was found that kidney altered in this way was much more nephritogenic than native kidney. Abnormal proteinuria in these groups developed between the 6th and 7th week and even rats that received only 6.4 mg of arsanil-sulfanil kidney had heavier proteinuria than rats given a full amount of native kidney in adjuvant (Table V). In addition, virtually 100% of rats developed disease after receiving the altered kidney. In the group immunized with undiluted altered antigen, one rat had only a moderate proteinuria but the others all had excretion of between 300 and 400 mg of protein a day. In the second group, all of the rats had a proteinuria in excess of 150 mg a day. The disease produced by chemically altered kidney was qualitatively identical morphologically to that produced by native kidney.

Discussion. The experiments reported here demonstrate that the course of kidney in adjuvant nephritis is influenced by alterations in any of the 3 variables, technique of immunization, host or antigen. In the experiments done to examine the role of adjuvant, streptococci were unable to replace *Mycobacterium tuberculosis*. However, replacing the emulsifier in the adjuvant, Arlacel C, with a more phlogogenic agent, Arlacel A, had an enhancing effect on the subsequent nephritis and proteinuria. This finding was of interest because it was possibly a manifestation of the direct nephrotoxic action of Freund's adjuvant(8).

Variations in immunization procedures also had considerable influence on the resultant nephritis. When Lewis rats were given a series of injections that contained $\frac{1}{5}$ the usual amount of kidney antigen, proteinuria appeared at the same time as when full doses of antigen were given, but was not well maintained. This was even more evident when the less susceptible Sprague-Dawley rats were immunized with $\frac{1}{3}$ the usual amount of antigen.

The importance of host factors in this disorder was also shown by these studies. For reasons that could not be determined, Buffalo

rats were apparently completely refractory to the production of nephritis. In contrast, the other 2 inbred strains tested, Lewis and Fisher, both developed more severe nephritis than did homologus Sprague-Dawley rats. However, the disease was morphologically similar in all 3 strains. The availability of 2 strains of rats that were both not only isologous, but also more susceptible to the induction of nephritis than the outbred strain originally used (1) makes the investigation of the pathogenesis of this disorder much easier.

In these studies efforts were directed to determining the inter-strain and inter-species distribution of the antigen, in an attempt to find the most effective way of producing disease. Our results showed that homologous kidney in adjuvant was the most efficient antigen in all 3 susceptible strains. They also indicated that the responsible factor was not exclusively a transplantation type of antigen, since isologous kidney in adjuvant caused disease in the Lewis strain of rats and produced a "forme fruste" in Fisher rats. Isologous kidney was, however, a much less efficient antigen than homologous kidney. This finding is not necessarily in disagreement with Heymann's report that autologous kidney produced nephritis as readily as homologous kidney(1). His observations were made on unilaterally nephrectomized animals and this procedure could well have increased vulnerability to nephritogenic agents. He did not have unilaterally nephrectomized rats as controls. Also, the discrepancy might be related to the fact that Sprague-Dawley rats were used by Heymann, and kidneys of this strain appear, in the present studies at least, to have the largest amount of the responsible antigen.

Chemical modification of the renal proteins by coupling them with arsanilic and sulfanilic acids appeared to give a more nephritogenic antigen. The reason for this increased effectiveness of the modified antigens is not known. It is possible that they are only quantitatively more effective than native antigens and act by similar means. Or they may induce immunologic responses different from those caused by native antigens, thereby invoking new pathogenic mechanisms. In any case, the increased immuno-pathogenicity of these tissue antigens caused by coupling with prosthetic groups is in agreement with the observations of Weigle using similar groups coupled to thyroglobulin(9).

Summary. An investigation was made into the importance of the immunization, strain of host rat, and antigen in the induction of kidney in adjuvant nephritis. It was found that variations in each of these 3 elements could significantly alter the development and course of the disease. Nephritogenic streptococci were ineffective substitutes for mycobacterium in the adjuvant. However, replacement of the usual emulsifying agent in the adjuvant, Arlacel C, with a more phlogogenic agent, Arlacel A, led to increased severity of disease. The strain of rat used was also important: inbred Lewis rats were most susceptible, inbred Fisher rats were next, outbred Sprague-Dawley rats were next and inbred Buffalo rats were refractory to development of nephritis. Several observations relating to the effectiveness of antigen were made. First, the most potent source of antigen appeared to be Sprague-Dawley kidneys. Second, isologous kidneys and heterologous kidneys provided less effective antigens than homologous kidneys. Third, it appeared that kidney antigen, in addition to stimulating an antibody response, had to be present in considerable quantity to induce a lasting renal injury. Fourth, multiple injections of kidney in adjuvant produced a more severe nephropathy than a single injection containing a comparable amount of kidney protein. This was possibly a manifestation of the increased nephrotoxic action of Freund's adjuvant in the former situation. Fifth, it was found that injections could be given at weekly instead of bimonthly intervals, thereby shortening the induction period of the disease by several weeks. Sixth, when a native kidney preparation was chemically altered by coupling it to diazonium derivatives of arsanilic and sulfanilic acid, and then used to immunize rats, a more severe disease was produced.

1. Heymann, W., Hackel, D. B., Harwood, S., Wilson, S. G. F., Hunter, J. L. P., Proc. Soc. Exp. Biol. and Med., 1959, v100, 660.

2. Hess, E. V., Ashworth, C. T., Ziff, M., J. Exp.

Med., 1962, v115, 421.

3. Heymann, W., Hunter, J. L. P., Hackel, D. B., Cuppage, F., Proc. Soc. Exp. Biol. and Med., 1962, v111, 568.

4. Heymann, W., Hunter, J. L. P., Hackel, D. B., J. Immunol., 1962, v88, 135.

5. Watson, J. I., Dixon, F. J., Fed. Proc., 1965, v24, 368.

6. Dixon, F. J., Unanue, E. R., Watson, J. I., IVth International Symposium on Immunopathology, eds., Pierre Grabar & Peter A. Miescher, Schwabe & Co., to be published.

7. Baker, M. C., Campbell, D. H., Epstein, S. I., Singer, S. J., J. Am. Chem. Soc., 1956, v78, 312.

8. Watson, J. I., Dixon, F. J., Feldman, J. D., Lab. Invest., 1965, v14, in press.

9. Weigle, W. O., J. Exp. Med., 1965, v121, 289.

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Blood Glucose and Corticosterone Changes Accompanying Altered Lipid Metabolism Induced by Exposure to Acceleration Stress. (30742)

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In studies reported earlier(1), some marked changes in fatty acid metabolism of tissues were noted in animals exposed to acute radial acceleration stress. These changes were expressed as an increase in the conversion of radioactive acetate to lipids by slices of liver, adipose tissue, and kidney from animals exposed to stress. The magnitude of the response was related to the age of the animal, to its nutritional status with regard to feeding or withholding food, to the duration of exposure time, and to G-load.

The experimental design of this early work involves *in vitro* techniques on tissues removed from the animals after exposure to stress. This procedure indicates residual hormonal or blood chemical changes produced by stress. Thus, observations on changes in levels of blood glucose or plasma corticosterone levels are essential for a better understanding of some of the sequential effects of exposure. The present study correlates blood component changes with metabolic and chemical changes in the tissues.

Methods. A 10 radial-armed centrifuge having an effective operating radius of 4.5 feet was employed. The cages were mounted in the swing-bucket fashion, allowing for one degree of freedom which placed the resulting G-vector perpendicular to the cage floor. The centrifuge was illuminated with fluorescent light automatically timed to provide on and off cycling at 6:00 a.m. and 6:00 p.m., respectively.

Nine-week-old Sprague-Dawley rats (225-250 g), maintained on Purina Laboratory Chow, were centrifuged at 4.7 *g* for varying time exposures. Noncentrifuged rats served as controls. Food was removed from the cages 24 hours prior to sacrifice. Hypophysectomized, adrenalectomized, and adrenodemedullated rats (Simonsen Laboratory, Gilroy, Calif.) were operated on when 6 weeks of age and were kept in the laboratory for 3 weeks prior to experiment. Hypophysectomized rats were given 5% glucose for drinking purposes during the first week after surgery and thereafter received whole-wheat bread, regular diet, and water. Adrenalectomized rats received 0.9% saline in place of water. Adrenodemedullated rats were maintained on regular diet. Plasma glucose(2) and plasma corticosterone(3) were determined on heparinized blood obtained from decapitated rats. Blood glucose(2) was determined on heparinized samples obtained from heart puncture performed under ether anesthesia.

Liver obtained from decapitated rats was cut into 0.5-mm slices with a mechanical tissue chopper(4). One gram of the slices was transferred to a 50-ml screw cap incubation flask(5) containing 10 ml of Krebs-bicarbonate buffer at pH 7.4. The medium contained