of control serum-treated sperm gave percentages of fertilization that approximated those obtained with the largest amounts of homologous antiserum-treated sperm. From these and from the other tests by extrapolation an estimate may be made of the reduction in fertilizing capacity of the sperm due to treatment with antiserum. This reduction ranges from 32-fold in 3 of the tests (Lytechinus 5 and 6, Urechis 1) to considerably more than 64-fold in the others. The difference apparently correlates with differences in antibody-titer (based on both original agglutinative titers and inhibitive titers) but more extensive tests would be necessary for the determination of any quantitative relation. The present results suffice to demonstrate that treatment of sperm with antisera can effect a greater than 64-fold reduction in fertilizing power.

In each of the tests the spermatozoa were examined microscopically for possible effects of antiserum on their motility. No noticeable differences from the activity of the spermatozoa in the control sera were observed, about 70 to 90% of the sperm being highly active in both.

Discussion. Since the antibodies employed in these tests were produced as a result of immunization of rabbits with antifertilizin obtained from the spermatozoa, it would appear that the inhibiting effect on fertilization furnishes further support of the view that the antifertilizin is intimately involved in the fertilization process. It does not, however, necessarily follow that the specific groups or structures of the antifertilizin molecules that may be involved in the initial reaction of sperm and egg are the same as those that serve as the active antigenic determinants in producing antibodies in the rabbit. It is quite conceivable that the antibodies are directed against other specific groups, and their presence on the surface simply blocks the action of those involved in the reaction with fertilizin, etc. Tests that are planned with closely related, difficultly cross-fertilizing, species may help decide this point.

In the present experiments the antibodies were first converted to a non-agglutinating "univalent," form in order to eliminate the mechanical effect of the clumping. It may be noted that such "univalent" antibodies may provide a useful tool in various types of immunological investigations involving enzymes, hormones, bacterial antigens, viruses, etc., where it is desirable to avoid the agglomerating action of the ordinary, "multivalent," antibodies.

Summary. Antisera were produced in rabbits by immunization with purified antifertilizin of Lytechinus and of Urechis and were photo-oxidized so as to convert the antibodies into the "univalent" (non-agglutinating) form. Sperm treated with such antisera were found to possess less than 1/64 of the fertilizing capacity of the controls although their activity was not noticeably affected. The bearing of the results on the significance of antifertilizin in the fertilization reaction is briefly discussed and the possible general use of "univalent" antibodies is mentioned.

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Influence of Amino Acid Feeding upon Antibody Production in Protein Depleted Rats.*

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(Introduced by Paul R. Cannon).

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In previous publications from this laboratory the deleterious effects of prolonged protein depletion upon antibody formation in rabbits and rats have been reported.[1,2] In

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man the association of famine and infectious disease[3] as well as the many examples of increased susceptibility to intercurrent infections evidenced by hypoproteinemic patients with chronic debilitating diseases[4] indicate a relationship between malnutrition and reduced resistance to infection. Furthermore, a correlation between a low gamma globulin level and an inability to form antibodies has been recently demonstrated.[5]

We have attempted to correct the reduced capacity to produce antibodies in protein depleted rats and have found that the feeding of adequate amounts of high quality protein will quickly lead to a restoration of the antibody producing mechanism.[6] Of particular interest was the finding that a casein hydrolysate (Amigen), when fed in adequate amounts, led to a marked improvement in antibody formation relative to that of hypoproteinemic rats fed a similar and isocaloric low protein ration in equivalent amounts. It appears likely, therefore, that restoration of the antibody forming mechanism resulted from the utilization of amino acids in the hydrolysate. This conclusion is strengthened by the following experiment in which crystalline amino acids were fed as the source of dietary amino nitrogen.

Experimental. The methods and rations used in inducing protein depletion in the rat, as well as the techniques of bleeding, serum protein and hemoglobin determinations, and hemolysin titration have been described.[2 6] In this experiment 3 comparable groups consisting each of 6 young adult male rats were

TABLE I.
Summary of Experimental Data.

No. of rats per group	Avg initial wt, (g)	Depletion period			Avg values at start of repletion period			Repletion period			Gain or loss after 7 days		
		Ration	Days	Avg wt. loss or gain, (%)	Avg wt, (g)	Avg serum prot., (g%)	Avg Hb., (g%)	Ration	Days before antigen inj.	Avg wt (g)	Avg serum prot., (g%)	Avg Hb., (g%)
6	212	3E	173	—35	137	4.64	10.5	4E	7	— 9	—0.38	—0.6
6	211	3E	173	—34	140	4.56	10.1	AAR	7	+12	+1.75	+3.7
6	212	3C	173	+52	319	7.13	16.2	4C	7	—15	—0.05	+0.2
6	208	3E	173	—35	136	4.52	9.4	AAR + liver	7	+14	+1.68	+3.8

Livestock and Meat Board, the Douglas Smith Foundation of The University of Chicago, and Allen B. Wrisley and Co.

[1] Cannon, P. R., Chase, W. E., and Wissler, R. W., *J. Immunol.*, 1943, **47**, 133.

[2] Cannon, P. R., Wissler, R. W., Woolridge, R. L., and Benditt, E. P., *Ann. Surg.*, 1944, **120**, 514.

[3] Cannon, P. R., *Sci. Monthly*, 1943, **56**, 5.

[4] Cannon, P. R., *J. Am. Diet. Assn.*, 1944, **20**, 133.

[5] Krebs, E. G., *J. Lab. and Clin. Med.*, 1946, **31**, 85.

[6] Wissler, R. W., Woolridge, R. L., Steffee, C. H., Jr., and Cannon, P. R., *J. Immunol.*, 1946, **52**, 267.

fed a low protein ration (diet 3E) for a depletion period of about 6 months, while an additional control group was fed a similar ration adequate in protein (diet 3C). After weighing these animals and determining their concentrations of serum protein and hemoglobin (Table I) one depleted group was placed on a practically protein-free ration (diet 4E; N x 6.25 = 0.88%). A second group received this same ration into which a mixture of 16 crystalline amino acids* modeled after the amino acid composition of casein was incorporated, by replacing an equivalent amount of cornstarch with 36.6 g of amino acids per 100 g of ration (diet AAR; N x 6.25 = 26.5). In addition the liver concentrate (Wilson & Co. 20:1) was omitted and the amounts of niacin, riboflavin, pyridoxine HCl, Ca Pantothenate and Choline Cl were increased in order to equalize the concentrations of these vitamins in the two rations. The third group of depleted rats was fed this second ration with the addition of 1% of liver concentrate (AAR plus Liver; N x 6.25 = 26.8). During this repletion period the control animals were fed a ration (diet 4C) which was similar to the low protein ration except that vitamin test casein was substituted for an equivalent amount of cornstarch (N x 6.25 = 21.4%). The animals were housed in individual wire-bottomed cages and their food intakes were carefully equalized by the paired feeding technique. Their daily diet consumptions averaged approximately 8.5 g per rat per day or about half the amount we have usually fed rats during the repletion period. After 7 days of repletion all the rats were weighed and bled for base line hemolysin titers as well as serum protein and hemoglobin concentrations. The average changes in weights, serum protein and hemoglobin concentration are summarized in Table I where it can be seen that the rats consuming the 4E ration continued to lose weight and concentrations of serum protein and hemoglobin while the rats fed the 4C ration lost weight but maintained a rather constant level of serum protein and hemoglobin. On the other hand the two groups fed the amino acid rations gained a small but significant amount of weight during the 7 days in spite of their restricted food intake. Simultaneously they made marked gains in serum protein and hemoglobin concentrations. There was no significant difference between the response of the rats receiving the amino acid ration without liver (AAR) as compared to the group receiving this ration with liver (Table I).

On the seventh day of repletion each animal was injected intravenously with 1.0 ml of 0.25% suspension of washed sheep erythrocytes. Blood for hemolysin titrations was obtained on the fourth, sixth and tenth days after injection. The average antibody curves are shown in Fig. 1. It is evident that the substitution of pure crystalline amino acids for carbohydrate with the other ingredients kept constant and the diet intakes equalized, caused a definite increase in antibody production, the average titers of the amino acid fed rats being from 4 to 5 times higher than those of the animals maintained on the low protein ration. Furthermore, there was little difference in antibody formation between the group which received liver concentrate and the group which did not. The average hemolysin titers of the control animals receiving diet 4C were considerably higher than those of any of these groups and averaged 340, 4160 and 768 on 4, 6 and 10 days respectively. As might be expected, the animals on the repletion regime had not regained their "normal" antibody producing capacity after only one week of re-feeding with amino acids when this was accompanied by a rather marked overall food restriction. It is probable that these animals were forced

* Grams of amino acids per 100 g of diet:

Arginine, l (+) HCl	1.536
Histidine, l (+) HCl · H_2O	1.046
Lysine, l (+) HCl · H_2O	2.935
Tryptophane, dl	0.558
Phenylalanine, dl	1.611
Methionine, dl	1.084
Threonine, dl	2.417
Leucine, l (—)	3.749
Isoleucine, dl	4.028
Valine, dl	4.338
Tyrosine, l (—)	1.983
Cystine, l (—)	0.112
Glutamic acid, l (—)	7.347
Aspartic acid, dl	1.952
Glycine	0.155
Alanine, dl	1.735
Total g amino acid/100 g diet	36.590

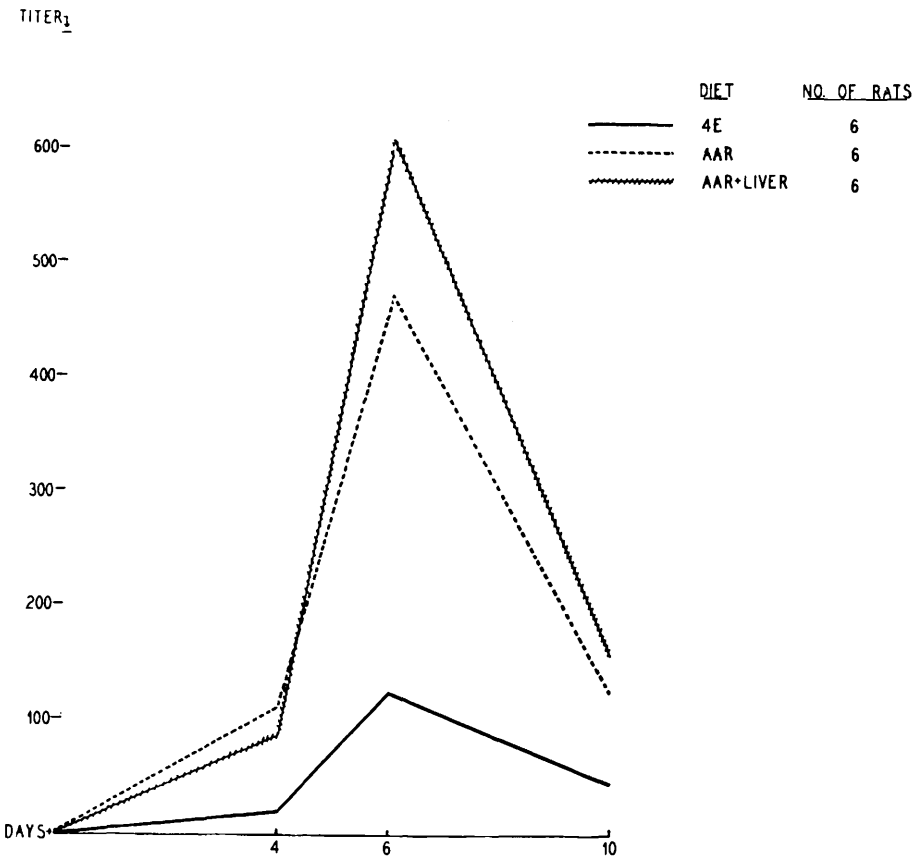

FIG. 1.
Average hemolysin titers at four, six, and ten days.

to utilize much of their ingested amino acids for energy metabolism. This probability is strengthened by the fact that in other experiments in this laboratory (to be published), protein depleted rats fed similar amino acid rations at a level of approximately 15 g per rat per day have consistently shown a weight gain of about 35 g in a 7 day period, even though the amino acid mixture was fed at a level of only 12.1 g per 100 g of diet. Furthermore, in investigating the relationship between caloric intake and the utilization of dietary protein we have found that the level of food consumption employed in this experiment markedly limits the depleted animal's weight gain and blood protein regeneration. Therefore, the results of this experiment probably do not represent the greatest degree of repletion of weight, serum protein, hemoglobin and antibody forming capacity which would be possible when amino acids are fed as the source of dietary amino nitrogen. These results do speak, however, for a recovery of a part of the lost capacity of protein depleted rats to fabricate antibody globulin when the only variable in the diets is the substitution of a mixture of amino acids for carbohydrate.

Others have reported recently that the growth of young animals is retarded when pure amino acids or completely hydrolyzed proteins are fed as the sole source of amino nitrogen, unless a factor (strepogenin) present in native proteins, liver concentrate or partially hydrolyzed protein is supplied.[7,8]

[7] Woolley, D. W., *J. Biol. Chem.*, 1946, **162**, 383.

[8] Womack, M., and Rose, W. C., *J. Biol. Chem.*, 1946, **162**, 735.

There was little evidence under the conditions of our experiment that the presence of factors in liver concentrate other than the vitamins mentioned above exert any appreciable effect upon the repletion of the depleted rat's tissue and blood proteins.

These results strengthen our view that antibody production depends upon an adequate supply of essential amino acids coming either from food or the animal's own protein stores. When both of these sources are markedly diminished the antibody producing capacity is markedly reduced. This capacity can be re-established by feeding adequate and well balanced quantities of amino acids coming from food protein, enzymatic protein hydrolysates, or amino acid mixtures.

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Increased Serum Phosphatase and "Hyperprothrombinemia" in Infectious Hepatitis of Children.

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The alkaline phosphatase activity of serum and the plasma prothrombin time are among the most commonly used indices of hepatic function. The phosphatase finds its most common application in the differential diagnosis of jaundice, where some observers believe the level of enzyme activity to be one of the most useful criteria in the differentiation of obstructive from hepatogenous jaundice.[1-3] Others,[4-8] while confirming that marked increases of phosphatase activity are more frequently found in obstructive than in "catarrhal" disorders of the liver, have observed overlapping to a considerable extent between the 2 groups. As to the behaviour of serum phosphatase in infectious hepatitis, results and conclusions of different observers are somewhat at variance. That some elevation of phosphatase occurs, appears evident from the figures of most studies.[1-9] While according to some, the increase of enzyme activity is only slight,[1-3] others[4-7,9] have frequently found greater rises. It has been suggested[1,2,5] that children may react during infectious hepatitis with higher phosphatase levels than adults, a suggestion in keeping with the results of one study, the subjects of which were for the most part children.[9]

In the study of the plasma prothrombin time, the greatest attention has been paid to the occurrence of hypoprothrombinemia. Lowering of the plasma prothrombin in patients with hepatic disease may be caused by a deficiency of vitamin K owing to decreased intestinal absorption or by impairment of the ability of the liver to form prothrombin. Two practical applications of the prothrombin time have been in the foreground: 1. as an index of bleeding tendency, and 2. as a

1 Roberts, W. M., *Brit. M. J.*, 1933, **1**, 734.

2a Flood, C. A., Gutman, E. B., and Gutman, A. B., *Arch. Int. Med.*, 1937, **59**, 981; 2b Gutman, A. B., Olson, K. B., Gutman, E. B., and Flood, C. A., *J. Clin. Invest.*, 1940, **19**, 129; 2c Gutman, A. B., and Hanger, F. M., Jr., *M. Clin. North America*, 1941, **25**, 837.

3 Rothman, M. M., Meranze, D. R., and Meranze, T., *Am. J. Med. Sc.*, 1936, **192**, 526.

4 Herbert, F. K., *Brit. J. Exp. Path.*, 1935, **16**, 365.

5 Shay, H., and Fieman, P., *Am. J. Digest. Dis.*, 1938, **5**, 597.

6 Giordano, A. S., Wilhelm, A., and Prestrud, M. C., *Am. J. Clin. Path.*, 1939, **9**, 226.

7 Greene, C. H., Shattuck, H. F., and Kaplowitz, L., *J. Clin. Invest.*, 1934, **13**, 1079.

8 Cantarow, A., and Nelson, J., *Arch. Int. Med.*, 1937, **59**, 1045.

9 Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 107.