rabbit hyper-immune serum. The immune serum was produced by repeated inoculations of rabbits with both spotted fever vaccine and the virus.[1] The intradermal route of inoculating guinea pigs with virus and serum was used.

Our study is based on the known phenomenon in which the infiltration of the skin in susceptible animals with specific immune serum affords protection against subsequent inoculation with the homologous virus of vaccinia.[2, 3, 4]

Three main factors were considered: the site of inoculation, the time factor and the amount of immune serum.

An area of ¼ square inch of guinea pigs' skin was infiltrated first with 0.4 cc of immune serum, and then two hours later 0.1 cc of virus was injected into this area. These animals remained afebrile and proved immune to subsequent reinfection with spotted fever.

Fifteen guinea pigs were then treated with 0.1 cc of virus and 0.4 cc immune serum simultaneously at the same site. No fever was noted in eleven of them, while four developed spotted fever. Only three of the non-reacting animals showed solid immunity to subsequent reinfection with spotted fever. Of six other guinea pigs injected with virus and serum as above but at *different sites* of the body, five developed typical spotted fever.

In the next series of thirteen guinea pigs smaller amounts of immune serum were used: 0.1 cc, 0.05 cc and 0.025 cc, the serum and 0.1 cc of virus given simultaneously and at the same site. Of these none developed spotted fever. Four weeks later they were all reinfected with a five-fold dose of virus and nine animals showed complete immunity in striking contrast with the control animals.

Inactivation of the virus in the skin was followed by solid immunity even when 0.025 cc of immune serum was injected at the same site and as long as 18 hours following the virus injection. Experiments are now under way to find out the lapse of time subsequent to virus inoculation after which immune serum will still protect the animal.

It is hard to believe that the negligible amount of 0.025 cc of immune serum could afford any degree of passive immunity. We may presume, however, that the virus absorbed by the highly potent anti-serum in the tissue acted as a sensitized vaccine. It is also possible that this amount of serum was not able to neutralize the virus completely, leaving a fraction of the virus to escape. Nevertheless, this negligible quantity of free virus would contribute to the protection of guinea pigs against the reinfection and thus confer a solid immunity. This interpretation clarifies the apparently puzzling phenomenon in which some of the guinea pigs treated with the same dose of virus (0.1 cc) but with larger amounts of immune serum (0.4 cc) did not develop immunity. Under these circumstances the virus was evidently rendered completely innocuous and the antigenic properties altered by the excess serum.

Whatever the intrinsic nature of these phenomena may be, they strengthen the emphasis laid originally by Besredka[5] on the role of the skin in infection and immunity. Due to certain autonomy of the skin as an organ it is possible by intradermal introduction of an attenuated pathogenic agent (bacterial or viral) to create a local immunity which is a step toward the production of a general immunity. In our work a local interception of the virus was achieved in various degrees by minute doses of specific anti-serum. Thus far the interception of the virus is still possible after 18 hours, but further experiments are in progress.

It will be of interest to determine whether it is possible to prevent the disease by the above method when the original tick virus is used.

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[1] N. H. Topping, *Publ. Health Rep.*, 55: 41, 1940.
[2] C. H. Andrewes, *Jour. Path. and Bact.*, 31: 671, 1928.
[3] R. W. Fairbrother, *Jour. Path. and Bact.*, 35: 35, 1932.
[4] R. H. Green and R. F. Parker, *Jour. of Immun.*, 45: 171, 1942.
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SCIENTIFIC APPARATUS AND LABORATORY METHODS

OBSERVATIONS ON THE BIOLOGICAL VALUE OF A MIXTURE OF ESSENTIAL AMINO ACIDS[1]

IN a series of convincing experiments, Rose[2] established the conception, now generally accepted, that only nine amino acids are essential for the growth of the rat, but that a tenth, arginine, must be supplied to attain maximal growth. The technique employed by him consisted in eliminating amino acids one at a time from a dietary mixture containing both essential and non-essential amino acids. Impairment of growth occurred only when one or another of the ten "essential" acids was eliminated.

[1] From the Department of Pediatrics, Johns Hopkins Hospital, Baltimore, Maryland. This investigation was aided by grants from the Rockefeller Foundation, Nutrition Foundation, Merck and Company and E. R. Squibb and Sons.

[2] W. C. Rose, *Physiol. Rev.*, 18: 109, 1938.

In addition to these classic studies, Rose stated in 1938[3] that animals whose only source of dietary nitrogen was a mixture of the ten essential amino acids "gained in weight just as rapidly as when all the protein components were supplied preformed." These latter experiments have never been published in detail. The question as to whether a mixture of essential amino acids provides an adequate substitute for dietary protein or complete protein hydrolysates has obvious practical implications. The recent study of Elman and Lischer[4] in dogs suffering from shock and that of Bauer and Berg[5] in mice have shown that a mixture containing only essential amino acids is distinctly inferior to a complete amino-acid mixture. In view of these results, it seemed desirable to us to carry out further experiments on rats in which the growth and nitrogen retention of animals whose sole source of N was an essential amino-acid mixture was compared with those of animals receiving complete amino-acid mixtures.

EXPERIMENTAL

Studies were carried out on young animals from a hybrid colony of albino and hooded Norwegian rats. Four diets were used which differed only in the composition of the nitrogenous moiety.[6] In diet "EAA," nitrogen was furnished as an essential amino-acid mixture[8] which differed, however, in some respects from that of Rose: the proportion of biologically "effective" amino acids used was that found in a typical animal protein—casein, and in addition a small amount of cystine was added. In diet "A," the nitrogenous moiety consisted of Amigen,[9] an enzymatic digest of casein in which approximately 15 per cent. of the nitrogen is derived from pancreatic protein. In diet "CTH," nitrogen was supplied as an acid hydrolysate of casein prepared in this laboratory which had been fortified with 1 per cent. cystine and 1.5 per cent. tryptophane, and in diet "C" it was supplied as unhydrolyzed casein. The food intake of animals on the "A," "CTH" and "C" diets was restricted to that of the animals on the essential amino-acid mixture.

RESULTS

The weight curves of animals on these different diets are shown in Fig. 1. The poorest performance was that of the animals on the "EAA" diet. These animals lost weight steadily and one of them succumbed during the second week. The general appearance of

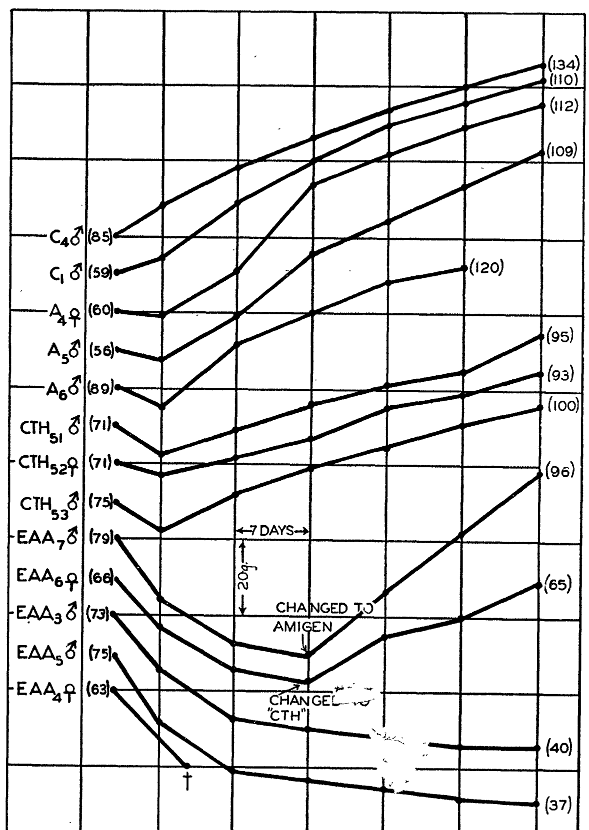

FIG. 1. Growth of rats on diets containing a crystalline amino acid mixture, reinforced acid hydrolyzed casein, Amigen and casein. The numbers in parentheses denote initial and final weights of the rats.

[3] Ref. 1, page 129.

[4] R. Elman and C. E. Lischer, *Jour. Am. Med. Asn.*, 121: 498, 1943.

[5] C. D. Bauer and C. P. Berg, *Jour. Nutr.*, 26: 51, 1943.

[6] The basal diet had the following composition:

Nitrogenous moiety	108.5 gms
(In the case of the essential amino-acid mixture, the quantity taken was 138.3 gms, allowance being made for the non-utilizability of the unnatural forms of certain amino acids added in the racemic form.)	
$NaHCO_3$	20.0 gms
Mineral mixture[7]	14.8 "
Sucrose	110.7 "
Starch	310.0 "
Agar	14.8 "
Cod liver oil	36.9 "
Crisco	140.0 "
Brewer's yeast	31.2 "

[7] W. M. Cox and M. Imboden, *Proc. Soc. Exp. Biol. and Med.*, 34: 443, 1936.

[8] Essential amino-acid mixture:

l-tryptophane	4.5 gms
l-lysine HCl	19.2 "
l-cystine	2.5 "
d-histidine HCl	6.8 "
d-arginine HCl	12.7 "
l-leucine	12.0 "
l-leucine—isoleucine mixture	14.0 "
dl-valine	32.0 "
dl-methionine	7.0 "
dl-phenylalanine	15.6 "
dl-threonine	12.0 "
	138.3 gms

[9] Kindly furnished by Mead Johnson and Company, Evansville, Ind.

these animals likewise was far from thriving. When a change was made to the Amigen ("A") diet or the fortified acid hydrolysate ("CTH") diet, gain in weight was promptly restored. The superior growth of animals on the Amigen diet as compared with the "CTH" diet may well be due to the pancreatic proteins present in Amigen, proteins which have recently been shown to have a high nutritive value.[10] The growth curves of animals on Amigen are comparable to those on casein with the exception of a slight lag when the animal is first put on Amigen.

Nitrogen balance studies were made on six additional animals which were fed the "EAA" diet. These studies were repeated after the diet had been changed to "CTH" diet. The data are shown in Table 1. It will be noted that although the diet on the "CTH" period was restricted to that taken on the "EAA" period, the animals gained weight during the "CTH" period in contrast to the loss of weight which occurred on the essential amino-acid diet. Despite the loss of weight on the "EAA" diet, the animals retained nitrogen, although the retention was noticeably less than that on the complete amino-acid diet. These experiments emphasize the fact that *in the growing animals* nitrogen retention can not be taken as a criterion of adequate nutrition.

TABLE 1

NITROGEN BALANCE OF RATS MAINTAINED ON THE AMINO ACID MIXTURE AND REINFORCED ACID HYDROLYZED CASEIN DIETS

Rat number and sex	Initial body weight	Amino acid mixture diet 4 × 3 day periods			CTH diet 4 × 3 day periods		
		Average daily food intake	Average N-balance per day	Weight change	Average daily food intake	Average N-balance per day	Weight change
	gms	*gms*	*mgms*	*gms*	*gms*	*mgms*	*gms*
EAA–10 ♂	60	4.2	+ 22.5	– 20	4.0	+ 31.1	+ 5
EAA–11 ♂	60	4.4	+ 29.7	– 17	4.0	+ 30.2	+ 4
EAA–12 ♂	60	4.1	+ 31.8	– 16	4.0	+ 42.0	+ 9
EAA–13 ♂	52	3.9	+ 29.2	– 15	4.0	+ 46.0	+ 8
EAA–14 ♂	61	3.45	+ 12.8	– 21	3.67	+ 36.0	+ 7
EAA–15 ♂	44	3.90	+ 19.8	– 10	3.92	+ 40.8	+ 7

Several possible explanations for the nutritive failure of the animals on the "EAA" diet suggest themselves. It is conceivable that the particular mixture employed contained too little of some one amino acid. In order to evaluate this possibility, a diet was prepared which contained essential amino acids in double the quantity used before (comprising 29.4 instead of 14.7 per cent. of the diet). Control experiments were carried out in which the nitrogenous moiety was supplied as "CTH" hydrolysate at twice the previous level. The food intake of the control animals was restricted to that of the "doubled EAA" group. The control animals on "double CTH" all survived, although their weight curves were flatter than those of the animals on 14.7 per cent. "CTH." On the other hand, the results on the high intake of essential amino acids were most unsatisfactory. Of six rats (54–60 gms) submitted to this diet, 3 ate well and died within 5 days, the other 3 ate the diet poorly and survived until sacrificed 3 weeks later. The weight loss in these animals was much more striking than in the 14.7 per cent. "EAA" animals.

It seems possible that the toxic effects of the high "EAA" diet as compared to the high "CTH" diet are brought about by the unnatural isomers of certain amino acids which can not be utilized by the animal. Bauer and Berg[5] attribute their unfavorable results with mice on a ten amino-acid diet to the "cost" of the additional synthetic work the organism is called on to perform—in energy and in building materials, undoubtedly factors of importance, but it is difficult to attribute the effects observed in our animals to other than a toxic cause. The possibility that one or more unutilizable optical forms of amino acids may exert a toxic influence is one that demands verification by direct experiment, and until it has been definitely excluded it would seem wise to employ only the natural forms in human therapy.

SUMMARY

(1) A mixture of "essential" amino acids, fed to rats, was found to be inferior from a nutritional point of view to enzymatic or acid casein hydrolysates or casein fed at comparable levels.

(2) Evidence is presented which suggests that the nutritive inadequacy of the essential amino-acid diet may be due in part to toxic effects of unnatural forms of certain amino acids that can not be utilized.

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