RELATION OF AMINO ACID IMBALANCE TO NIACIN-TRYPTOPHANE DEFICIENCY IN GROWING RATS*

By W. A. KREHL, L. M. HENDERSON, J. DE LA HUERGA, AND C. A. ELVEHJEM

(From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison)

(Received for publication, July 11, 1946)

The growth inhibition which results from the inclusion of corn in a low protein, niacin-deficient ration (1) and the apparent dual rôle of niacin and tryptophane in counteracting this growth depression have resulted in considerable study on the mechanism of these relationships. It has been indicated that intestinal microorganisms may play a significant part in this deficiency syndrome, since the deleterious effect of corn can be greatly modified by the use of carbohydrates previously shown to favor intestinal synthesis. The suggestion has also been made that corn might contain a substance structurally analogous to niacin which would compete with this vitamin (2). The observation that tryptophane administration results in the increased excretion of niacin and niacin derivatives (3) indicates that the similar growth-promoting activity of niacin and tryptophane may be due to the fact that tryptophane functions as a metabolic precursor of niacin. The fact that niacin synthesis is greater on a synthetic ration than when corn is added, despite the fact that the former ration contains slightly less tryptophane than the latter (1), does not lend credence to the explanation that tryptophane acts as a precursor of niacin under these conditions. It has been demonstrated, however, that niacin may improve the animal's production or utilization of tryptophane (4). If the synthesis

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by grants from the National Dairy Council, on behalf of the American Dairy Association, the Nutrition Foundation, Inc., and the Wisconsin Alumni Research Foundation. Dr. J. de la Huerga was supported by a fellowship from the Institute of International Education, 2 West 45th Street, New York 19, New York, and by the University of Santo Domingo, Dominican Republic.

We are indebted to Merck and Company, Inc., Rahway, New Jersey, for the supply of crystalline B vitamins, to the Abbott Laboratories, North Chicago, Illinois, for the generous supply of haliver oil, and to the Lederle Laboratories, Inc., Pearl River, New York, for the synthetic *Lactobacillus casei* factor.

The 3-acetylpyridine hydrochloride and the pyridine-3-sulfonic acid used in these experiments were supplied by Dr. F. M. Strong, Biochemistry Department, and Dr. S. M. McElvain, Chemistry Department, University of Wisconsin, respectively.

of niacin is impaired or if niacin is supplied in inadequate amounts, the dietary requirement for tryptophane is increased.

Considerable evidence is at hand to show that the chief protein of corn (*i.e.*, zein), other proteins such as gelatin, and acid-hydrolyzed proteins, free of tryptophane, create a syndrome exactly analogous to that produced by corn (5). These results indicate that the growth depressions caused by corn and the non-corn-containing rations which contain tryptophane-free proteins have a common basis; namely, an imbalance of amino acids.

Attempts have been made in the present study to extend our information concerning the deleterious action of corn grits and the counteraction by various materials and to elucidate the nature of the amino acid imbalance conducive to the growth failure with rations which contain added protein or protein hydrolysates free of tryptophane.

EXPERIMENTAL

The composition of the diets used is given in Table I. The vitamins were added in the following amounts per 100 gm. of ration: thiamine 0.2 mg., riboflavin 0.3 mg., pyridoxine 0.25 mg., calcium pantothenate 2.0 mg., choline 100 mg., 2 methyl-1,4-naphthoquinone 0.1 mg., inositol 10 mg., and biotin 0.01 mg. Vitamins A and D were supplied as halibut liver oil, 2 drops every week, together with α-tocopherol, 1 mg. per rat per week. Unless otherwise indicated, the basal rations contained practically no nicotinic acid. Weanling male rats of the Sprague-Dawley strain were used in all cases and each group contained three rats.

In an effort to determine whether an insoluble sulfonamide would inhibit the effect of niacin in corn grits rations, sulfasuxidine was added with and without niacin. Synthetic *Lactobacillus casei* factor was also added at a level of 25 γ per 100 gm. of ration, since a deficiency of this factor is known to occur in the rat in the presence of sulfonamides (6). The results for Groups 1 and 2 in Table II show that sulfasuxidine does not counteract the beneficial action of niacin. In a similar experiment, *streptothricin* was added to the corn grits ration at a level of 25,000 units per 100 gm. of ration and it likewise failed to impair the beneficial action of niacin. If it is assumed that niacin favors the intestinal synthesis of tryptophane it appears that the above drugs did not impair the activity of organisms responsible for this synthesis. This is not surprising in view of the fact that these drugs have been shown to alter the synthesis of some but not all factors.

In an experiment to test the value of natural foodstuffs in counteracting the action of corn grits two rations were devised containing peanuts and brewers' yeast respectively. These rations were prepared by reducing the casein content so that the tryptophane level was approximately the same as

that in the usual corn grits ration. In this manner the effectiveness of these supplements could be tested, based on their niacin content, which for the amounts used was about 1.5 mg. per cent for both the peanut and brewers' yeast rations. The results shown in Table II, Groups 3 and 4, indicate the beneficial value of these supplements in the corn grits diet.

In a report on the nutritive value of the Mexican tortilla (7), it was indicated that this food, made from corn, provided a major portion of the Mexican dietary. Pellagra, while not unknown in Mexico, does not appear extensively as a result of this diet. Tortillas were prepared,

TABLE I

Composition of Basal Ration

Ration constituent	Ration No.								
	1	2	3	4	5	6	7	8	9
Sucrose	78	84		38	43		82	82	82
Dextrin			84			73			
Casein*	15	9	9	7	8	9	9	9	9
Gelatin						6			
Zein						3			
Fibrin hydrolysate						2	2		
Casein "								2	
Egg albumin hydrolysate									2
Peanuts (ground)				10					
Brewers' yeast					4				
Corn grits				40	40				
" oil	3	3	3	2	2	3	3	3	3
Salts IV†	4	4	4	3	3	4	4	4	4
l(−)-Cystine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

* Crude casein extracted three times with boiling 95 per cent ethanol.

† Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, **109**, 657 (1935).

therefore, according to the method described by Cravioto (7), dried, and added to the basal ration in the usual manner. It can be seen that dried tortillas inhibited growth just as severely as corn grits (Table II, Group 6) and that niacin completely counteracted this inhibition. Since the most significant difference between the Mexican tortilla and whole yellow corn is the much higher calcium content of the tortilla, the yellow corn ration was supplemented with 0.5 per cent $CaCO_3$. Marked growth inhibition was again evident (Table II, Group 5), which was corrected with niacin.

In a previous report (1) it was shown that, of the various cereals tested, only corn inhibited growth under the conditions used. Additional experiments with cow peas, which are used extensively in the South, and with

TABLE II

Growth Results on Various Diets

Group No.	Basal ration (Table I)*	Type of ration† and supplement to basal ration	Gain per wk. for 4 wks., 3 rats per group‡
			gm.
1	1	Corn grits 40%	9
2	1	" " 40 % + 2% sulfasuxidine	4 (+N, 29)
3	4	(" " 40%, peanut)	31
4	5	(" " 40%, brewers' yeast)	29
5	1	Yellow corn, 40% + $CaCO_3$, 0.5%	7 (+N, 30)
6	1	Dried tortillas 40%	9 (+N, 27)
7	1	Cow peas 40%	28
8	1	Corn bran 40%	30
9	1	" grits 40% + 5% corn germ	28
10	1	" " 40% + 10% " "	25
11	1	" " 40% + 3% dried pig stomach	27
12	1	" " 40% + 3-CN pyridine 1.5 mg. %	7 (+N, 32)
13	1	" " 40% + indole 125 mg. %	4 (+T, 33)
14	1	" " 40% + anthranilic acid 100 mg. %	7 (+T, 33)
15	1	" " 40% + indole-3-acetic acid 100 mg. %	4 (+T, 31)
16	2	(Sucrose)	15 (+N, 18)
17	3	(Dextrin)	27
18	2	(Sucrose) + pyridine-3-sulfonic acid 0.2%	9 (+N, 15)
19	2	" + 3-acetylpyridine · HCl 0.2%	17
20	6	(Dextrin-casein 9%, gelatin 6%, zein 3%, fibrin hydrolysate 2%)	11 (+N, 32) (+T, 29)
21	3	(Dextrin) + glycine 1%	25
22	3	" + *l*(−)-leucine 1%	24
23	3	" + *l*(+)-glutamic acid 1%	23
24	3	" + glycine, *l*(−)-leucine, and *l*(+)-glutamic acid 1% each	27 (+N, 27) (+T, 24)
25	2	(Sucrose) + glycine 1.5%, *l*(−)-leucine 1%	8 (+N, 18)
26	2	" + *l*(+)-glutamic acid 1.5%, *l*(−)-leucine 1%	12 (+N, 21)
27	2	(Sucrose) + glycine 1.5%, *l*(−)-leucine 1.0%, *l*(−) tyrosine 0.25%, *dl*-phenylalanine 0.5%, *dl*-valine 0.25%	5 (+N, 13)
28	2	(Sucrose) + *l*(+)-glutamic acid 1.5%, *l*(−)-tyrosine 0.25%, *dl*-phenylalanine 0.5%, *dl*-valine 0.25%	11 (+N, 16)

* Supplements of corn grits, yellow corn, dried tortillas, cow peas, and corn bran were added at the expense of the entire basal ration indicated. Other supplements were added at the expense of sucrose, except 3-CN pyridine, indole, anthranilic acid, indole-3-acetic acid, pyridine-3-sulfonic acid, and 3-acetylpyridine hydrochloride which were added to the entire basal ration.

† Parentheses indicate the type of ration.

‡ The letters N and T in parentheses indicate oral supplementation with 0.25 mg. of niacin and 10 mg. of *dl*-tryptophane per rat per day respectively. The accompanying figures are the average weekly gains during the 4th and 5th weeks when supplements were given. The figures outside of the parentheses represent average weekly gains during the first 3 weeks. A control rat from each group was continued on the deficient or niacin-low ration.

corn bran show that these materials fed at a level comparable to corn grits produce no growth inhibition (Table II, Groups 7 and 8).

The excellent nutritive quality of corn germ is evident by the fact that when it was included in the corn grits ration at a level as low as 5 per cent (at the expense of sucrose) good growth was obtained. In considering the somewhat surprising effectiveness of such low levels of corn germ it is worth while to note the narrow range in which the level of tryptophane is active in counteracting growth inhibition. For example when wheat gluten and gelatin were used as the protein source (1) and the tryptophane content was 120 mg. per cent, poor growth resulted, but the same ration allowed good growth when the tryptophane level was increased to only 145 mg. per cent. The effectiveness of corn germ may be due in part to the fact that it contributes several other of the amino acids which may be present in the corn grits ration in borderline amounts, although its protective action is perhaps better explained by its higher niacin (*i.e.* about 40 to 45 γ per gm.) and tryptophane content (see Table II, Groups 9 and 10). Dried pig stomach, a good source of both niacin and tryptophane, gave excellent protection against corn grits (see Table II, Group 11).

Certain compounds related to niacin or tryptophane were tested for their ability to counteract the growth deficit caused by corn grits. It has been shown that 3-CN pyridine is unable to cure canine blacktongue (8) and it is clear that it has no niacin activity in the rat, although when niacin was given after 3 weeks to rats receiving 3-CN pyridine a marked growth response was observed (Table II, Group 12). Indole and indole-3-acetic acid were tested for tryptophane activity in counteracting the deleterious effect of corn and found to be completely inactive. No competitive activity of these compounds against tryptophane was observed since added *dl*-tryptophane, at a level of 10 mg. per rat per day, resulted in excellent growth. Anthranilic acid was tested in a similar manner because it is known to be active for certain bacteria (9), but it too was inactive, although it did not prevent the beneficial action of tryptophane. (The results are given in Table II, Groups 13 to 15).

Woolley reported the antiniacin activity of certain compounds related to niacin and showed that 3-acetylpyridine given orally in single test doses created a niacin deficiency in the mouse (10) which was counteracted by large doses of niacin. In a similar experiment with pyridine-3-sulfonic acid, no niacin inhibition was observed even at very high levels of this compound (11).

In the present study 3-acetylpyridine as the hydrochloride was incorporated in the ration at a level of 0.2 per cent with no untoward effects (compare Group 19 with Group 16, Table II). Whether this difference is due to species variation or the somewhat different manner in which the

compound was given is uncertain. When pyridine-3-sulfonic acid was included in the same ration at a level of 0.2 per cent (9 per cent casein, sucrose basal) some growth retardation was observed which was counteracted by niacin (Table II, Group 18). In earlier experiments this compound had been fed to rats at levels up to 3 per cent with no harmful effects. However, at that time we were using a ration containing 20 per cent casein. This difference in behavior of the sulfonamide may be largely due to the difference in the protein level of the rations. Woolley has shown, for example, that the antiniacin action of 3-acetylpyridine can be counteracted with tryptophane (2).

In a continuation of the previous study with non-corn, low protein rations (5) experiments were conducted to study the growth inhibition caused by the addition of proteins or protein hydrolysates free of tryptophane. In verification of previous results (5), dextrin produced better growth than sucrose when the basal ration contained 9 per cent casein (Table II, Groups 16 and 17). However, the presence of dextrin did not protect the rat from the growth inhibition which resulted when tryptophane-free proteins or protein hydrolysates, such as a combination of 6 per cent gelatin, 3 per cent zein, and 2 per cent acid-hydrolyzed fibrin, were added (Table II, Group 20), despite the fact that the nitrogen content was increased to a level corresponding to about 20 per cent protein.

That the growth inhibition induced by acid-hydrolyzed proteins such as fibrin, egg albumin, and casein at levels of 2 per cent in a 9 per cent casein ration can be immediately counteracted by niacin, even after 3 weeks on the inhibitory regimen, is evident in Fig. 1, growth Curves B, C, and D. The control rats on these rations which received no niacin grew very little. It is also clear that when niacin is added the slopes of the growth curves are the same as those obtained with rations containing no supplement of acid-hydrolyzed protein (Fig. 1, Group A). The addition of niacin to the latter ration stimulates growth only slightly.

Inasmuch as one or more of the amino acids, glycine, leucine, and glutamic acid, are present in rather large amounts in the tryptophane-low proteins, zein and gelatin, and in the tryptophane-free acid-hydrolyzed proteins, fibrin and casein, these amino acids were added separately and collectively at a level of 1 per cent each to the 9 per cent casein-dextrin ration. No growth inhibition could be observed from these amino acids under the conditions employed (Table II, Groups 21 to 24). When sucrose was used as the carbohydrate, however, and glycine and *l*(−)-leucine added together at a level of 1.5 and 1 per cent respectively, a growth inhibition was observed which was corrected by the addition of niacin at a level of 250 γ per rat per day. A combination of glutamic acid and leucine at levels of 1.5 and 1 per cent respectively produced slightly less growth than that

obtained with the 9 per cent casein-sucrose basal, but the addition of niacin resulted in marked growth stimulation. The addition of 0.25 per cent *l*(−)-tyrosine, 0.5 per cent *dl*-phenylalanine, and 0.25 per cent *dl*-valine to the above combinations of glycine and *l*(−)-leucine seemed to depress growth still more and the addition of niacin was much less effective in

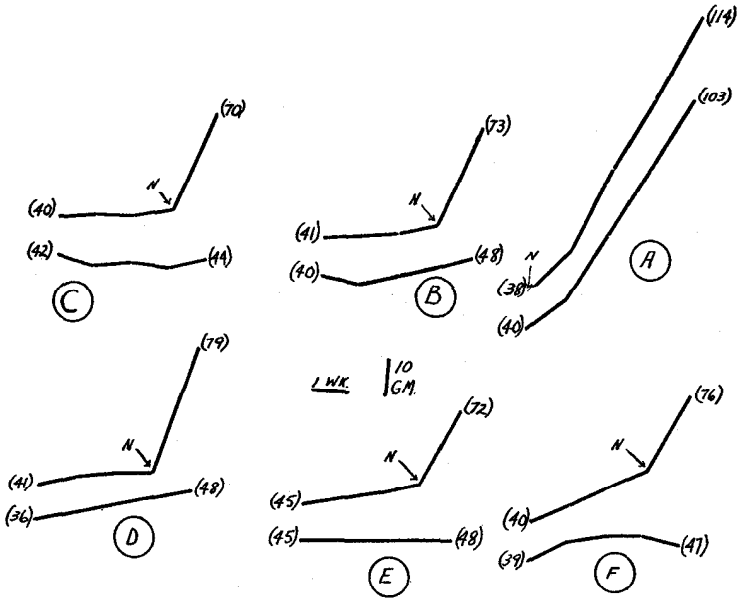

FIG. 1. Growth responses in rats on various diets when supplemented with niacin (*N*) at the indicated point in the growth curve. Group A, 9 per cent casein (sucrose) basal with and without niacin; Group B, 9 per cent casein (sucrose) basal plus 2 per cent acid-hydrolyzed fibrin; Group C, 9 per cent casein (sucrose) basal plus 2 per cent acid-hydrolyzed egg albumin; Group D, 9 per cent casein (sucrose) basal plus 2 per cent acid-hydrolyzed casein; Group E, 9 per cent casein (sucrose) basal plus 6 per cent gelatin; and Group F, 9 per cent casein (sucrose) basal plus 2 per cent glycine.

counteracting this inhibition (for the results consult Table II, Groups 25 to 28).

The growth inhibition caused by 6 per cent gelatin and the counteraction by niacin are shown in Fig. 1, Group E. That glycine is intimately concerned in the amino acid imbalance involved in these studies is indicated by the fact that the addition of 2 per cent glycine to the sucrose-casein ration tends to retard growth (Fig. 1, Group F) and niacin counteracts this retardation. It should be noted, however, that glycine caused very little, if any, growth inhibition when dextrin was used as the carbohydrate.

One of the observations made upon autopsy of rats which received corn

without added niacin was the fact that the livers appeared fatty and friable. When the fat content of these livers was determined, it was found that the animals which received corn had, as a rule, more liver fat than the animals receiving added niacin. On analysis of the livers from the deficient animals for niacin (12) it was found that they contained less of this vitamin than the control animals. That the level of choline has a bearing on the problem of liver fat is evident from the fact (Table III)

TABLE III

Niacin and Fat Content of Rat Livers on Various Diets

Basal ration (Table I)	Supplement to basal ration*	Liver niacin				Liver fat	
		−N†	+N†	−T‡	+T‡	−N†	+N†
		γ *per gm.*	γ *per gm.*	γ *per gm.*	γ *per gm.*	*per cent*	*per cent*
1	(Sucrose basal) (choline 100 mg. %)	160	165			3.0	2.8
1	+ 40% corn grits	110	164			8.4	3.2
1	+ 40% yellow corn (choline 60 mg. % in ration)	116	146			19	3.3
1	+ 40% corn grits + indole-3-acetic acid			80	136		
1	+ 40% " " + 3-CN pyridine	84	108				
1	+ 40% " " (choline 10 mg. % in ration)	84	120			25	20
1	+ 40% corn grits (choline 30 mg. % in ration)	86	86			22	18
1	+ 40% corn grits (choline 60 mg. % in ration)	110	126			17	9.7
2	(Sucrose) + 3% gelatin	98	105		159		
2	" + 3% zein	77	108				
2	" + 2% acid-hydrolyzed fibrin	97	108		141		

* The parentheses indicate the type of ration.

† −N and +N refer to no niacin in the ration and added niacin at a level of 1.5 mg. per cent.

‡ −T and +T refer to no added tryptophane or added tryptophane at a level of 50 mg. per cent.

that, as the choline content of the ration is decreased, the liver fat markedly increased, even though the animals still received a favorable growth response when niacin was added. However, the niacin content of the liver remained low as long as liver fat was high.

It was interesting to note (Table III) that the niacin content of the livers from deficient animals which received the growth-inhibiting proteins, gelatin and zein, or acid-hydrolyzed fibrin was low and was not greatly increased until the addition of niacin. When tryptophane was added, however, the content of niacin in the liver markedly increased. This would indicate that tryptophane may play a direct rôle in niacin synthesis (3).

DISCUSSION

The evidence so far obtained indicates that the action of corn in niacin-low synthetic rations is a function of the character of the predominant protein present, zein, and that this, in turn, is due to an amino acid imbalance in this protein. It seems that this imbalance is made apparent through growth depression under any set of circumstances which provides too little tryptophane and niacin. That an imbalance of amino acids results in an increased tryptophane requirement, and therefore poor growth, is evident by the fact that rats will grow reasonably well on rations which contain the same amount of tryptophane but which also contain a well balanced protein. This increased requirement could, however, be related to a greatly reduced supply of niacin, through inadequate synthesis, which results in decreased tryptophane utilization or production.

It is clear that the kind of carbohydrate used in the ration influences the results obtained. The very much better growth obtained with dextrin as compared with sucrose even on the basal ration probably indicates one of three things: (1) dextrin is stimulating the synthesis of additional factors needed by the rat; (2) dextrin not only provides desirable conditions in the intestinal tract for bacterial activity but, because of its slow hydrolysis and absorption, favors a more gradual absorption and better utilization of other accompanying nutrients; and (3) dextrin may carry some unidentified factor *per se*. A combination of the first two postulations seems more reasonable.

Albanese and Irby (13) early reported the inferiority of essential amino acids for growth as compared with protein hydrolysates and implicated the unnatural forms of certain amino acids for this growth failure. Kinsey and Grant (14) indicated that high levels of glycine were toxic and Hier *et al.* (15) also showed that gelatin and the amino acids, glycine, phenylalanine, and proline, resulted in growth inhibition. The levels used in these instances were undoubtedly toxic since adequate amounts of niacin were included in their rations.

Although glycine of the amino acids tested seems to inhibit growth the most, this cannot be the only amino acid involved, since acid-hydrolyzed casein and zein, both of which inhibit growth, contain little or no glycine. This does not preclude the possibility that more than one amino acid may be involved in the imbalance of amino acids in such circumstances.

SUMMARY

Natural materials, such as peanuts, brewers' yeast, corn germ, corn bran, and dried pig stomach, used as supplements to the corn grits ration under controlled conditions, adequately prevent growth inhibition.

Compounds such as 3-CN pyridine, indole, indole-3-acetic acid, and anthranilic acid did not replace either niacin or tryptophane in counter-

acting the deleterious action of corn grits, but neither did they compete with niacin or tryptophane to reduce their beneficial action.

Some inhibition of growth, which was corrected by niacin, was observed by pyridine-3-sulfonic acid in rations containing a low level of protein but not at a high protein level.

An amino acid imbalance in zein and other tryptophane-low proteins creates a growth depression on niacin-low diets, and, of the specific amino acids so far tested, glycine seems to be one of the amino acids most responsible. This effect is dependent to some extent on the type of carbohydrate used, since glycine exhibits no inhibition when dextrin is used in place of sucrose. In all cases, dextrin rations produced markedly superior growth to sucrose rations.

BIBLIOGRAPHY

1. Krehl, W. A., Sarma, P. S., Teply, L. J., and Elvehjem, C. A., *J. Nutr.*, **31**, 85 (1946).
2. Woolley, D. W., *J. Biol. Chem.*, **162**, 179 (1946).
3. Rosen, F., Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, **163**, 343 (1946).
4. Krehl, W. A., de la Huerga, J., and Elvehjem, C. A., *J. Biol. Chem.*, **164**, 551 (1946).
5. Krehl, W. A., Sarma, P. S., and Elvehjem, C. A., *J. Biol. Chem.*, **162**, 403 (1946).
6. Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, **145**, 713 (1942).
7. Cravioto, R. O., Anderson, R. K., Lockhart, E. E., Miranda, F. P., and Harris, R. S., *Science*, **102**, 91 (1945).
8. Woolley, D. W., Strong, F. M., Madden, R. J., and Elvehjem, C. A., *J. Biol. Chem.*, **124**, 715 (1938).
9. Snell, E. E., *Arch. Biochem.*, **2**, 389 (1943).
10. Woolley, D. W., *J. Biol. Chem.*, **157**, 455 (1945).
11. Woolley, D. W., and White, A. G. C., *Proc. Soc. Exp. Biol. and Med.*, **52**, 106 (1943).
12. Krehl, W. A., Strong, F. M., and Elvehjem, C. A., *Ind. and Eng. Chem., Anal. Ed.*, **15**, 471 (1943).
13. Albanese, A. A., and Irby, V., *Science*, **98**, 286 (1943).
14. Kinsey, V. E., and Grant, W. M., *Science*, **99**, 303 (1944).
15. Hier, S. W., Graham, C. E., and Klein, D., *Proc. Soc. Exp. Biol. and Med.*, **56**, 187 (1944).