betes for a preliminary period of 2 to 4 months, was given a diet with excess nicotinic acid. The diet was adequate but contained 1 g niacin/kg. The most outstanding effect of this diet was the production of ketonuria in 16 of the 20 animals, although the degree of ketonuria and the response pattern was variable. Supplementary choline did not inhibit ketosis. The action of nicotinic acid in altering fat metabolism is discussed.

15619

Inadequacy of Certain Natural Diets for Guinea Pigs.*

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Ross, Phillips and Bohstedt[1] found that a ration composed of corn, soybean oil meal and alfalfa, which is similar to rations widely used for growing hogs, is inadequate for reproduction and lactation in swine and rats. It was, therefore, of interest to try this ration with guinea pigs in order to determine if the deficiencies in these animals were related to the postulated factors required specifically by guinea pigs.

Experimental and Results. The methods of handling the animals and securing the blood samples have been described previously.[2] Young guinea pigs initially weighing 150 to 200 g were housed in individual screen-bottomed cages, and kept on experiment for 6 weeks.

The basal ration (R-1) consisted of ground yellow corn 75 g, soybean oil meal 17.5 g, ground alfalfa 5 g, and salts 2.5 g. ($CaHPO_4$ 1.18 g, $CaCO_3$ 0.70 g, NaCl (iodized) 0.60 g, $MnSO_4$ 0.03). When supplements were incorporated into the ration, the amount of corn was correspondingly decreased. The letter *c* following the ration number indicates that the ration contained crystalline vitamins in the amounts used in experiments with purified diets:[2] thiamine 0.3 mg, riboflavin 0.5 mg, pyridoxine 0.4 mg, Ca *d*-pantothenate 1.5 mg, niacin 5 mg, choline chloride 100 mg, biotin 0.02 mg, *i*-inositol 50 mg, and 2-methyl-1,4-naphthoquinone 0.2 mg. All animals were given 20 mg of ascorbic acid orally every other day and 1 mg of α-tocopherol and 0.05 cc of haliver oil orally twice a week. The solution of α-tocopherol in corn oil was mixed with the haliver oil just prior to feeding.

The results obtained with Ration R-1 with and without various supplements are summarized in Table I. The results for the group receiving stock ration, a commercial-pelleted guinea pig feed, were described in a preceding publication[2] and are included for comparison. The animals that received Ration R-1 grew very poorly and less than half of them survived the 6-week experimental period. These animals showed a moderate anemia and leucopenia.

The addition of the crystalline vitamins (R-1c) enabled all animals to survive the experimental period and to maintain essentially normal levels of blood elements with a rate of growth ⅓ to ½ that of animals receiving the stock ration. With the work of Krehl, Teply, Sarma and Elvehjem[3,4] in mind, the vitamin mixture was replaced by

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1 Ross, O. B., Phillips, P. H., and Bohstedt, G., *J. Animal Sci.*, 1942, **1**, 86; 1942, **1**, 353.

2 Cannon, M. D., Mannering, G. J., Zepplin, M., Elvehjem, C. A., and Hart, E. B., *Arch. Biochem.*, 1945, **7**, 55.

3 Krehl, W. A., Teply, L. J., and Elvehjem, C. A., *Science*, 1945, **101**, 283.

4 Krehl, W. A., Teply, L. J., Sarma, P. S., and Elvehjem, C. A., *Science*, 1945, **101**, 489.

TABLE I.
Growth and Blood Status of Guinea Pigs Fed a Natural Ration, With and Without Various Supplements.

Ration	No. of animals	Growth: Avg, g per day for 6 wks	Growth: Range, g per day for 6 wks	Blood*: Leucocytes, per mm3	Blood*: Hemoglobin, g per 100 cc
Stock ration	15	7.5	5.0-10.2	5300	14.2
R-1	7	1.3	—1.1- 3.3	3900	11.8
R-1 + 0.01% niacin	4	2.7	1.8- 3.8	5400	12.1
R-1 + vitamin mixture (R-1c)	12	3.0	1.8- 5.1	6200	13.0
R-1c + 4% liver 1:20 powder	6	3.8	2.5- 6.6	7400	13.8
R-1c + 4% grass juice powder	3	3.9	3.1- 5.0	5700	13.1
R-1c + 15% extract wheat germ	3	4.2	4.1- 4.3	4700	12.5
R-1c + 10% linseed oil meal	3	4.8	4.1- 5.6	7100	11.2
R-1c + 25% ” ” ”	6	6.5	5.0- 7.5	7500	14.1
R-1c + ethanol extract l.o.m. ≅ 25%	3	3.5	2.7- 4.2	2900	12.5
R-1c + 25% l.o.m. residue	3	4.5	3.4- 5.4	8600	13.5
R-1c + l.o.m. extr. + residue ≅ 25%	3	6.7	5.4- 7.3	4700	13.5
R-1c + 25% soybean oil meal	3	5.6	5.0- 6.1	5300	12.8
R-1c + 8% Labco casein	3	4.9	3.6- 6.8	4700	14.3

* After 6 weeks on experiment.

a high level of niacin. Although growth and survival were better than on Ration R-1 alone, neither the growth nor the blood status was equal to that of animals provided with much smaller amounts of niacin, together with other vitamins.

The inclusion of 4% of 1:20 liver powder† or 4% of grass juice powder‡ in Ration R-1c gave little additional improvement in growth or blood formation. Extracted wheat germ (VioBin) at a level of 15% gave a small but definite improvement in growth, as did linseed oil meal at a 10% level. When 25% of linseed oil meal was added, blood formation was excellent and growth approached that of the animals fed the stock ration. Extraction of linseed oil meal with cold 50% ethanol (extract contained 3% of original l.o.m. solids) appears to remove some active substance, since growth with the rations containing the residue alone was inferior to that with whole linseed oil meal. The extract appeared ineffective by itself but when it was recombined with the residue growth as good as that with whole linseed oil meal was obtained. The addition of 25% of soybean oil meal (a total of 42% in the ration) supported good growth, although the average growth and hemoglobin values were lower than with linseed oil meal at 25%. The inclusion of 8% of Labco casein in Ration R-1c gave a definite average growth increase, though there was individual variation from 3.6 to 6.8 g per day. This average increase is probably significant since only 3 out of 12 animals on Ration R-1c alone showed gains in excess of 3.4 g per day. The blood picture was uniform and essentially normal.

The foregoing data indicate that Ration R-1 supplemented with vitamins A, D, E, and C is inadequate for growth and blood formation in guinea pigs. The deficiency may be made good in part by a mixture of the crystalline water-soluble vitamins of which niacin appears to be one, but not the only, active component. The blood status of animals receiving the vitamin supplement approaches that of animals fed the stock ration, although growth is still poor. Cunha *et al.*[5] have shown that added inositol is required with this ration to prevent alopecia in the rat and that inositol together with pyridoxine and a folic acid concentrate in-

† Dried crude water extract of liver.

‡ Grass juice powder is the spray-dried pressed juice of cereal grasses. One g of grass juice powder is equivalent to about 4 g of dried grass (Cerophyl).

5 Cunha, T. J., Kirkwood, S., Phillips, P. H., and Bohstedt, G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 236.

TABLE II.
Effect of Tryptophane as Compared with Other Supplements on Growth and Blood Status of Guinea Pigs.

Ration	No. of animals	Growth: Avg, g per day for 6 wks	Growth: Range, g per day for 6 wks	Blood*: Leucocytes, per mm^3	Blood*: Hemoglobin, g per 100 cc
Stock ration	13	5.2	3.0-6.7	6900	14.1
R-1c	8	1.9	0.5-2.8	5700	13.4
R-1c + 0.3% *dl*-tryptophane	4	3.4	3.0-3.8	7300	14.4
R-1c + ethanol extract l.o.m. ≅ 25%	4	2.0	0 -3.5	6000	13.2
R-1c + ethanol extract l.o.m. ≅ 25% + 0.3% *dl*-tryptophane	4	3.0	1.6-4.8	6600	14.2
R-1c + 25% l.o.m. residue	4	4.1	2.6-6.1	5400	13.9
R-1c + l.o.m. residue + extr. ≅ 25%	4	4.1	3.1-5.0	5100	14.5

* After 6 weeks on experiment.

creases the growth rate of the rat. Folic acid does not appear to be concerned here since 1:20 liver powder and grass juice powder, both rich in this factor, give only slight gains. The specific guinea pig factors postulated by ourselves and others[2,6,7] to be present in these materials are ruled out by this same evidence.

The substances which produced the greatest growth stimulation, linseed oil meal, soybean oil meal, and casein are protein-rich materials. Wheat germ which gave a slight response is likewise a good source of protein; 15% supplying 6% additional protein. The action of these substances does not appear to be due to the increase in the quantity of protein, since this is presumably adequate in the basal (Ration R-1, 15%, stock ration 17%), but to the addition of amino acids or other essential factors present only in limited quantity in Ration R-1. The studies of Krehl *et al.*[3,4] suggest that tryptophane may be a limiting factor with diets high in corn.

Assays employing *Lactobacillus arabinosus*§ indicate that 100 g of Ration R-1 contains 0.20 g of tryptophane. Similar determinations show that the inclusion of 8% of casein supplies 0.10 g, 25% of soybean oil meal, 0.17 g, and 25% of linseed oil meal, 0.24 g of additional tryptophane per 100 g of ration. Calculations from the tables of Block and Bolling[8] indicate that 15% of wheat germ, which gave relatively little growth stimulation, adds only 0.06 g of tryptophane. Thus the growth stimulating potency of these 4 supplements appears to be directly proportional to their tryptophane content. The most effective of these, linseed oil meal, essentially doubles the amount of tryptophane in the ration. Apparently little or no tryptophane is destroyed or extracted from linseed oil meal by cold 50% ethanol since the residue contains 10.0 mg per g and the extract 2 mg per g (original linseed oil meal 9.5 mg per g).

In view of the above results we decided to test the effect of adding pure tryptophane. Unfortunately, no animals from our stock colony were available so it was necessary to use guinea pigs purchased from a commercial breeder. The animals as received from the breeder were given stock ration for a few days, then distributed at random among the groups receiving the R-rations listed in Table II. A group of animals from this same breeder received stock ration and the results are also included for comparison. All animals showed a slower rate of growth, regardless of the ration fed. This is made clear by comparing the growth of the groups fed stock ration in Tables I and II.

6 Woolley, D. W., *J. Biol. Chem.*, 1942, **143**, 679.

7 Woolley, D. W., and Sprince, H., *J. Biol. Chem.*, 1944, **153**, 687.

§ The authors are indebted to Irene Greenhut, through whose cooperation these determinations were made.

8 Block, R. J., and Bolling, D., *The Amino Acid Composition of Proteins and Foods.*

The addition of 0.3% of *dl*-tryptophane to Rations R-1c and R-1c + linseed oil meal extract stimulates growth and possibly increases the hemoglobin level (Table II) although it does not seem to duplicate completely the effect of 25% of linseed oil meal residue plus extract. In this connection it is interesting to point out that Woolley and Sprince[7] have shown that the dietary essential for guinea pigs designated at GPF 2 and found in linseed oil meal residue can be replaced by a mixture of cellulose and casein or cellulose, arginine, cystine and glycine. Apparently the guinea pig is susceptible to certain amino acid deficiencies and the limiting amino acid is dependent upon the kind of ration used. Since we have obtained excellent growth and development on a stock ration containing only 17% total protein it is evident that the amino acid requirement of guinea pigs is easily influenced by the remaining components of the diet.

Linseed oil meal extract does not seem to potentiate the action of tryptophane, nor in this series did it increase the growth stimulus supplied by 25% of linseed oil meal residue. Although growth on the linseed oil meal supplemented rations was much poorer than in the series reported in Table I, it should be noted that the addition of linseed oil meal does allow growth almost equal to that of animals from the same source receiving stock ration.

Summary. Guinea pigs fail to grow on a corn-soybean oil meal-alfalfa ration even when adequate amounts of vitamins A, D, E, and C are supplied. The survival and blood picture of the animals are improved by the addition of a mixture of crystalline water soluble vitamins of which niacin appears to be an important, but not the only, active component. Linseed oil meal, soybean oil meal, and casein supplements promote growth and blood formation approaching that of animals on stock ration. The potency of these substances is proportional to their tryptophane content and may be replaced in part by pure tryptophane.

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15620 P

Effects of Androgen upon Reproductive Organs of Normal and Castrated Fetuses with Note on Adrenalectomy.*

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This report deals with progress of studies designed to determine whether in placental mammals the normal, prenatal development of the reproductive organs is influenced by hormones. Recently it has been found that in unborn rats the number and size of the interstitial cells of Leydig may be increased experimentally by injecting gonadotrophin under the skin of the unborn.[1]

In the present experiments the animals used were rats in which pregnancy was dated from the time of observation of copulation. Fetuses were killed at about one hour preceding the estimated time of normal parturition (21 days and 16 hours after copulation). After weighing a fetus, its pelvic region was fixed in Bouin's solution and sectioned serially at 10 μ. The sections were stained with hematoxylin and eosin or with

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1 Wells, L. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 250.