of the protein of bread is available for the growing rat and that fortification with lysine increases this to about 60 percent. Flodin's statement (*4*), "there is a latent store of protein in bread and flour that needs only one key to release it for the body's use. That key is lysine fortification" should be amended to at least three keys, lysine, threonine, and methionine.

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Zinc Requirement of the Chick

Over 20 years ago it was found that the growth of young rats was greatly impaired if their diet was extremely low in zinc. Keratinization of the skin, parakeratosis of the esophagus, loss of hair, and decreased activity of intestinal phosphatase, blood phosphatase, and carbonic anhydrase were later associated with zinc deficiency in the rat (*1*). Zinc has also been shown to be required by the mouse (*2*) and pig (*3*).

Recently, O'Dell and Savage (*4*) reported that the growth of chicks was stimulated if zinc was added to a semipurified diet containing 25 percent of Drackett assay protein C-1. No response to zinc was obtained if casein or alpha protein replaced the Drackett protein or if the level of potassium in the ration was low (*5*). All rations contained about 50 ppm of zinc. The chicks were brooded in batteries which ordinarily are zinc coated, and tap water was supplied, presumably in the galvanized water troughs of the batteries.

We have also studied this problem with chicks (*6*) but have attempted to reduce substantially the amount of zinc ingested. This was done in several ways: (i) selecting ingredients low in zinc; (ii) extracting the zinc from the protein supplement (the source of much of the zinc of the basal ration chosen); (iii) coating the batteries or building nonmetallic pens to prevent contamination with this element; (iv) conducting experiments in isolated quarters to reduce dust as a possible source of zinc; (v) using distilled water instead of tap water; and (vi) providing the drinking water in glass rather than galvanized troughs. All these changes were intended to reduce the amount of zinc ingested by the birds.

In an exploratory experiment, two groups of 5-day-old, White Leghorn cockerels were allocated to pens having plastic mesh sides and rubber mesh floors. The basal diet, similar to that used by Morrison *et al.* (*7*) without the zinc supplement, was fed to one group. This basal diet contained about 16 ppm of zinc. The second group was supplied the basal ration supplemented with 100 ppm of zinc as the sulfate, added by way of the salt premix. The chicks were allowed to eat *ad libitum* in glass feeders, and distilled water was supplied in Pyrex glass waterers.

The group which received the low-zinc, basal diet grew poorly, developed dermatitis of the feet, failed to feather properly, and developed a goose-stepping walk. Symptoms began to appear about the 14th day and were severe by the 21st day. The group fed the zinc-supplemented ration grew satisfactorily during the 4-week test period and did not exhibit any of the symptoms noted in the zinc-deficient group.

On the basis of the results of this preliminary study, a more comprehensive experiment was designed. In this, the Drackett protein was extracted by washing with a hydrochloric acid solution at *p*H 4.6 and then dried about 36 hours in an oven at temperatures up to 100°C. The unextracted basal ration contained 19 ppm of zinc; the extracted basal ration analyzed 7 ppm (*8*). Basal rations containing either the unextracted or extracted protein were fed by themselves and with 100 ppm of zinc as zinc sulfate. A total of 120 White Meteor × White Rock 1-day-old chicks were allotted on the basis of weight into the four groups, each of which contained three replicates of five females and five males each.

The experimental birds were brooded in an electrically heated battery with raised, wire floors. All battery parts were coated with epoxy plastic; the bottoms of the dropping pans and wire floors were coated with shellac. Glass founts having a plastic base supplied distilled water for drinking. The battery was isolated in a room relatively free from dust.

The basal diet was essentially that used by Morrison *et al.* (*7*) without the zinc supplement. Drackett protein was used in basal diet 1; the extracted and dried product was used in basal diet 2. Minerals were reagent-grade chemicals. The experimental diets were mixed in plastic equipment and stored in plastic bags. Experimental diets and distilled water were supplied *ad libitum* throughout the experimental period. Feed consumption was recorded.

Table 1. The effect of zinc deficiency and supplemental zinc on chick weights and feed conversion.

Group	Treatment (diet)	Av. wts* at 4 wk (g)	Gain in wt per lb of feed (lb)
1	Basal 1	183	0.45
2	Basal 1 + 100 ppm zinc	363	0.69
3	Basal 2	101	0.35
4	Basal 2 + 100 ppm zinc	333	0.62

* Significantly different. Orthogonal comparison (*9*). $P < .01$ for groups 1 and 3 versus 2 and 4; group 1 versus group 3; $P < .05$ for group 2 versus group 4.

As shown in Table 1, the birds in groups 1 and 3 grew poorly and utilized their feed inefficiently. As in the previous test, they developed a severe dermatitis of the feet, frizzled wing feathers, infantile body feathers, and a goose-stepping walk.

Addition of 100 ppm of zinc to the ration containing the unextracted protein (group 2) produced normal growth, feathering, and feed utilization. When zinc was added to the ration containing the extracted protein (group 4), response was not quite as good as it had been on the unextracted protein. This difference in performance between groups 2 and 4 shows that some factor(s) in addition to zinc was removed by extraction or that the process of extraction and drying altered the nutritive value of the protein supplement.

The results of these experiments indicate that zinc is required by the chick for growth, feather development, and maintenance of a healthy condition of the skin of the feet as well as for the efficient utilization of feed.

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Demonstration of the 3-Methoxy Analog of Norepinephrine in Man

Recent studies by Armstrong, McMillan, and Shaw (*1*) have shown that a major metabolite of norepinephrine and epinephrine found in human urine is 3-methoxy-4-hydroxymandelic acid. Subsequently it was shown by Axelrod (*2*) that the 3-hydroxy position of both norepinephrine and epinephrine can be methylated by animal tissues to yield the corresponding 3-methoxy analogs. The 3-methoxy analog of epinephrine (ME) was also reported to be present in rat urine (*2*). Since the conversion of catecholamines to their methoxy analogs may play some role in the physiology of these agents, it seemed important to determine whether these substances exist in man.

Table 1. Chromatographic behavior of methoxy analogs of catecholamines and the compound extracted from four pheochromocytomas. Abbreviations correspond to the methoxy analogs of norepinephrine (MN), epinephrine (ME), and 3,4-dihydroxyphenylethylamine (MD). The chromatograms were sprayed for phenols with a freshly prepared solution of N, 2,6-tri-chloro-*p*-quinoneimine (0.05 percent weight/volume in ethanol) followed by borate buffer, *p*H 9.0 (*4*).

Compound	R_f		Color
	Isopropanol-5% NH_3 (8:2)	Butanol-acetic acid-water (8:1:1)	
1	0.62–0.66	0.31	Blue
MN	0.65	0.30	Blue
ME	0.80	0.28	Blue
MD	0.77	0.40	Tan-Pink

Since preliminary attempts to demonstrate the methoxy amines in human urine were unsuccessful, it was decided to look for them in tissues. A human tumor tissue which can be readily obtained and which contains large amounts of the parent catecholamines is pheochromocytoma. When several such tumors were extracted and the extracts subjected to paper chromatography, they were found to contain appreciable quantities of the 3-methoxy analog of norepinephrine (MN).

The tumors (*3*) were homogenized in 6 volumes of 0.01*N* HCl, adjusted to *p*H 10 by addition of a saturated solution of sodium carbonate, and extracted three times with 5 volumes of n-butanol. To the combined butanol extracts were added an equal volume of heptane and 0.2 volume of 0.5*N* HCl. After shaking, the aqueous layer was evaporated to dryness *in vacuo* and the residue taken up in 10 ml of methanol. The methanol was then evaporated to 0.4 ml, and portions were subjected to chromatography on Whatman No. 1 paper with two different solvents. The results of these studies, summarized in Table 1, indicate that all the tumors contained a butanol extractable base (compound 1) identical with MN, as shown by its R_f and the color obtained with the phenol reagent and ninhydrin. In several instances the areas corresponding to MN on the chromatograms obtained with isopropanol-NH_3 were eluted and rechromatographed with butanol-acetic acid. The R_f values obtained were again identical with those of authentic MN. The tumor containing epinephrine was found also to contain two additional phenolic substances, compounds 2 and 3. Compound 2 appeared on the chromatograms between MN and ME and gave a bluish-green color with the phenol reagent, unlike that of the three available standards. Compound 3 exhibited R_f values and color (tan-pink) identical with those of 3,4-dihydroxyphenylethylamine (MD).

The areas on the chromatogram corresponding to compounds 1, 2, and 3 were eluted with 0.01*N* HCl, and their fluorescence characteristics were compared with those of authentic MN, ME, and MD, by means of an Aminco-Bowman spectrophotofluorometer (Fig. 1). All three compounds and the reference standards exhibited maximal activation at 280 mμ and maximal fluorescence at 330 mμ.

Further evidence about the identity of compound 1 was obtained by eluting it from the chromatogram and subjecting it to distribution between equal volumes of 0.5*M* borate buffer, *p*H 10, and n-butanol. Analyses were carried out spectrophotofluorometrically. The distribution coefficients (concn. in butanol/concn. in aqueous) obtained for compound 1 and MN were 0.43 and 0.44, respectively,

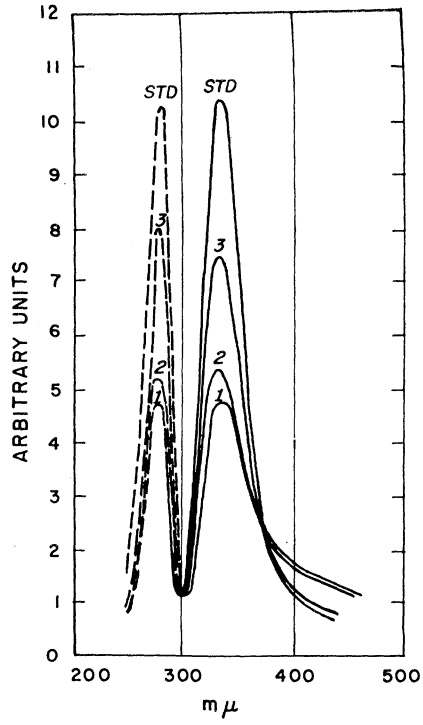

Fig. 1. Activation and fluorescence spectra in 0.01*N* HCl. Compound 1, apparent MN; compound 2, unknown; compound 3, apparent MD, Std., authentic MN; spectra obtained with authentic ME and MD were identical with those of MN.

whereas values for ME and MD were 4.0 and 6.1.

One tumor subjected to quantitative assay for MN by means of a combination of paper chromatography and spectrophotofluorometry was found to contain about 25 μg/g. Preliminary studies indicate that the tumors contain the enzyme which transfers the methyl group from S-adenosylmethionine to the 3-hydroxy group of catecholamines (*5, 6*).

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6. A more complete study of the biochemistry of catecholamines in pheochromocytoma is in preparation.

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