SUMMARY

1. Spores of *Aspergillus niger* in a glucose medium developed normally in presence of sodium arsenite at concentrations varying from 0.001 to 0.004 M, but germination was inhibited at 0.010 M.

2. Preformed mycelial felts of *A. niger* treated with arsenite at concentrations 0.001, 0.002, and 0.004 M, respectively, subsequently consumed more sugar, accumulated more felt material and produced a greater total of acids (citric, oxalic and gluconic) in a given time than did controls in absence of arsenite. Enhancement of metabolic activity was greatest at 0.004 M.

3. In presence of 0.010 M arsenite sugar consumption by preformed felts was not appreciably greater than it was in absence of arsenite, while increase in felt weight was less than in the arsenite-free control. Formation of acids in presence of 0.010 M-arsenite was greater than in the control, but less than it was at 0.004 M. Arsenite at 0.020 M almost completely suppressed the mould's activities.

4. Arsenite at all concentrations between 0.001 and 0.010 M reduced carbon dioxide output. This effect was greatest at 0.004 M.

5. The bearing of these results on the mode of formation of citric acid is discussed.

REFERENCES

- Butkevich, V. S. (1922). *Biochem. Z.* **131**, 327.
 Butkevich, V. S. (1924). *Biochem. Z.* **154**, 177.
 Chughtai, M. I. D., Pearce, A. A. & Walker, T. K. (1950). *Biochem. J.* **47**, 135.
 Hockenhull, D. J. D. (1950). *Biochem. J.* **46**, 605.
 Krebs, H. A. (1933a). *Hoppe-Seyl. Z.* **217**, 191.
 Krebs, H. A. (1933b). *Hoppe-Seyl. Z.* **218**, 157.
 Krebs, H. A. & Johnson, W. A. (1937). *Enzymologia*, **4**, 148.
 Szent-Györgyi, A. (1930). *Biochem. J.* **24**, 1723.
 Walker, T. K. (1949). *Advanc. Enzymol.* **9**, 537.
 Willstätter, R. & Schudel, G. (1918). *Ber. dtsh. chem. Ges.* **51**, 780.

Studies on the Biochemistry of Zinc

1. EFFECT OF FEEDING ZINC ON THE LIVER AND BONES OF RATS

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The indispensability of zinc as an element in nutrition has now been fairly established. Todd, Elvehjem & Hart (1934), Stirn, Elvehjem & Hart (1935), Day & McCollum (1940) and Day & Skidmore (1947) were able to show that when animals were fed a diet deficient in zinc they grew subnormally and developed various symptoms which were cured by supplementing the diet with small amounts of zinc, but attempts to elucidate the mechanism of its action have not been very successful. Hove, Elvehjem & Hart (1937) showed that deficiency of zinc resulted in the impairment of intestinal absorption. Other changes brought about by a deficiency of this element have been reported by Gorter (1939) and Day (1942). Scott (1934) and Scott & Fisher (1935, 1938a, b) showed that even the purest preparations of insulin contain zinc and that this element is somehow associated with the hypoglycaemic action of insulin. Keilin & Mann (1939) reported the presence of this element as a component of the enzyme, carbonic anhydrase, but Hove, Elvehjem & Hart (1940a, b) and Day & McCollum (1940) could find no change in the carbonic anhydrase activity in cases

of zinc deficiency. Davidson (1942) and Holmberg (1939) reported the presence of zinc in their preparations of uricase, but Wachtel, Hove, Elvehjem & Hart (1941) could not observe any difference in the uric acid content of the blood of normal and deficient animals. Further work is therefore necessary to find out how this element functions as an indispensable constituent of food of animals.

In this and the following papers, the problem has been attacked from a standpoint somewhat different from that of the investigations mentioned above. Here, the changes brought about by zinc when fed in concentrations much higher than the optimum have been investigated since it was observed in the preliminary experiments of the author that in broad outline, the changes brought about by either a deficiency of zinc or by toxic concentrations of it were almost identical. Moreover, since the preparation of diets completely deficient in this element without affecting any of the other constituents in the diet is very laborious and not quite satisfactory, it was thought that the changes brought about by the element when fed in toxic concentrations would be easier to follow and would lead eventually to a better elucidation of the biochemical functions of

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this element. The process may be regarded as analogous to the elucidation of the nature of activity of an enzyme by an investigation of its activation and inhibition.

Investigations on the changes brought about by diets containing excess zinc have been few. Grimmett, McIntosh, Wall & Hopkirk (1937) reported that, on a high-zinc diet, pigs showed toxic symptoms in about 2 months. This toxicity syndrome was characterized by loss of appetite and stiffness of the legs. On autopsy, the liver showed necrosis and the head of the humerus was affected. They also reported that zinc accumulated in the pancreas, kidney, liver and humerus. Sutton & Nelson (1937) observed that high-zinc diets brought about anaemia, subnormal growth and reproductive failure in rats. This was confirmed by Smith & Larson (1946) who investigated the nature of the anaemia caused by zinc. According to them, it was microcytic and hypochromic, and the condition could be cured by additional supplements of copper while supplements of liver or liver extracts caused resumption of growth.

During the present investigation, it was observed that the high-zinc diets brought about a reduction in the weight and also a reduction in fat content of the liver. Zinc appeared to have a lipotropic effect. Development of the bones was also adversely affected. A preliminary report of this work has already appeared (Sadasivan, 1950).

EXPERIMENTAL

Albino rats of the Haffkine inbred strain were divided into groups of six, with an equal distribution between the sexes

in each group. All the rats were fed a basal diet consisting of the following (parts by weight): wheat (*Triticum vulgare*), 58; gram (*Cicer arietinum*), 12; peas (*Pisum sativum*), 12; rice (*Oryza sativa*), 6; green leaves (*Amaranthus* sp.), 6; milk, 6. The constituents, excepting the green leaves and milk, were ground to a powder and well mixed. Weighed quantities of the powder were worked into a dough with the proportionate quantities of milk and green leaves, steamed for 30 min. and fed to the animals *ad lib*. On this diet, rats have been observed to thrive well. The supplements of Zn were made as pure grade ZnO (Merck) at levels of 0.5 and 1.0% in groups 2 and 3 respectively. The experiment extended over a period of 15 days only, since a longer period resulted in the death of rats in group 3. The food intake for each 3-day period was noted and growth measurements made at the end of each 3-day period, and, at the close of the experiment, the animals were killed by ether and liver, kidneys and femurs removed for assay. The fresh weight of liver and kidneys was noted after superficial drying with filter paper. The livers were minced and extracted with acetone in a Soxhlet extractor to determine the fat content. The results are presented in Table 1.

The femurs were cleaned of adhering flesh by rubbing with cloth, extracted with ether-ethanol mixture and dried to constant weight. They were ashed in a muffle furnace after preliminary heating with a gas burner and the ash weight noted. The ash was dissolved in pure dil. HCl, made up to a volume of 100 ml. and Ca and P determined in measured portions of the solution. Ca was determined by precipitating it as the oxalate and titrating it against standard permanganate solution. P was determined by the method of Fiske & Subbarow (1925) and the results are summarized in Table 2.

It will be observed from these tables that, on a stock diet, supplements of ZnO lowered the growth rate, the weight of liver and its fat content. The addition of ZnO to the diet at an 0.5% level did not affect the food intake, but the higher

Table 1. *Analysis of livers of rats fed on a stock diet supplemented with zinc*

(Figures in brackets represent standard deviation.)

Group	Diet	Initial wt. (g.)	Final wt. (g.)	Average daily food intake (g.)	Fresh wt. of liver (g.)	Wt. of liver (as % of final wt.)	Fat in liver (g.)	Fat in liver (as % of liver wt.)	Fresh wt. of kidney (g.)
1	Stock diet	96.0	138.0	21.4 (±0.59)	5.585 (±0.13)	4.050 (±0.10)	0.3844 (±0.010)	6.89 (±0.58)	1.230 (±0.03)
2	Stock diet + 0.5% ZnO	102.0	139.5	21.7 (±0.81)	4.823 (±0.17)	3.485 (±0.18)	0.2935 (±0.016)	5.88 (±0.24)	1.195 (±0.04)
3	Stock diet + 1.0% ZnO	103.0	116.0	19.0 (±0.64)	4.458 (±0.04)	3.865 (±0.17)	0.2707 (±0.030)	6.07 (±0.64)	1.160 (±0.014)

Table 2. *Analysis of femurs of rats fed a stock diet supplemented with zinc*

(Figures in brackets represent standard deviation.)

Group	Diet	Final body wt. (g.)	Dry wt. of femurs (g.)	Ash wt. (g.)	Ash (%)	Ca (mg.)	P (mg.)	Ca/P ratio
1	Stock diet	138.0	0.3875 (±0.009)	0.1990 (±0.0065)	51.35 (±0.94)	68.28 (±2.14)	40.35 (±0.80)	1.692 (±0.066)
2	Stock diet + 0.5% ZnO	139.5	0.3248 (±0.028)	0.1553 (±0.0153)	47.52 (±1.27)	54.51 (±5.39)	33.30 (±2.84)	1.637 (±0.030)
3	Stock diet + 1.0% ZnO	116.0	0.2608 (±0.011)	0.0998 (±0.0098)	38.09 (±2.65)	32.63 (±3.52)	25.05 (±1.91)	1.303 (±0.046)

Table 3. *The analysis of livers of rats fed on a high-fat, low-protein diet supplemented with zinc*

(Figures in brackets represent standard deviation.)

Group	Diet	Initial wt. (g.)	Final wt. (g.)	Average daily food intake (g.)	Fresh wt. of liver (g.)	Wt. of liver (as % of body wt.)	Fat in liver (g.)	Fat content of liver (%)
1	10% casein, 30% fat diet	50	63	7.7	5.26 (± 0.43)	8.35 (± 0.56)	1.2775 (± 0.296)	24.29 (± 3.90)
2	As group 1 + 0.5% ZnO	47	53	6.3	4.04 (± 0.16)	7.62 (± 0.22)	0.5727 (± 0.118)	14.18 (± 2.27)
3	As group 1 + 1.0% ZnO	45	47	5.1	3.03 (± 0.16)	6.45 (± 0.19)	0.3687 (± 0.080)	12.17 (± 1.96)
4	10% casein, 10% fat diet	53	76	9.6	6.08 (± 0.27)	8.00 (± 0.15)	1.0367 (± 0.127)	17.05 (± 1.60)
5	As group 4 + 1.0% ZnO	48	47	6.9	2.78 (± 0.23)	5.96 (± 0.23)	0.1420 (± 0.039)	5.11 (± 1.24)

Table 4. *Analysis of bones of rats fed on a high-fat, low-protein diet supplemented with zinc*

(Figures in parentheses represent standard deviation.)

Group	Diet	Final body wt. (g.)	Dry wt. of femurs (g.)	Ash wt. (g.)	Ash (%)	Ca (mg.)	P (mg.)	Ca/P ratio
1	10% casein, 30% fat diet	63	0.2430 (± 0.008)	0.0898 (± 0.0075)	36.95 (± 0.56)	34.8 (± 0.79)	14.70 (± 1.58)	2.53 (± 0.31)
2	As group 1 + 0.5% ZnO	53	0.2195 (± 0.009)	0.0643 (± 0.0027)	29.29 (± 0.64)	24.4 (± 1.21)	11.00 (± 1.07)	2.22 (± 0.20)
3	As group 1 + 1.0% ZnO	47	0.1863 (± 0.009)	0.0512 (± 0.0022)	27.48 (± 1.34)	18.6 (± 0.84)	8.55 (± 0.44)	2.21 (± 0.16)
4	10% casein, 10% fat diet	76	0.2477 (± 0.009)	0.1120 (± 0.0028)	45.22 (± 0.53)	40.8 (± 0.99)	19.52 (± 0.32)	2.09 (± 0.04)
5	As group 4 + 1.0% ZnO	47	0.1563 (± 0.011)	0.0502 (± 0.0055)	32.12 (± 1.32)	17.6 (± 2.30)	8.74 (± 1.07)	2.01 (± 0.04)

level of supplement markedly decreased the intake of food. The analysis of the femurs of rats showed that Zn appreciably reduced the dry weight and ash content of the bones. Evidently, the supplement interfered with development and mineralization.

The analysis of livers presented in Table 1 shows that Zn may act as a lipotropic agent though this fact is not unequivocally demonstrated by the results obtained on rats fed a stock ration. In order to confirm this point, Zn was added to a high-fat, low-protein diet on which rats normally developed fatty livers in about 15 days. Rats about a month old and of weight ranging between 40 and 60 g. were divided into five groups of six rats each. The first three groups were fed a diet consisting of ethanol-washed casein (choline content 1.5 mg./100 g.) 10 parts, hydrogenated groundnut oil 30, salt mixture (McCollum's) 4, sucrose 55 and shark-liver oil (12,000 i.u. vitamin A/g.) 1 part. In addition, the diet contained 15 μ g. each of aneurin, riboflavin, and pyridoxine hydrochloride, 50 μ g. of niacin and 20 μ g. of calcium pantothenate/g. Groups 4 and 5 were fed a diet similar to the above but with only 10 instead of 30% fat, the difference being made up with sucrose. Groups 2, 3 and 5 received supplements of ZnO at 0.5, 1.0 and 1.0% levels, respectively. The low-fat groups (groups 4 and 5) were included in the series in order to find out the extent to which Zn proved toxic or lipotropic, as the case may be, when the fat content of the diet was low. The rats were

weighed every 4th day and the food intake for each 3-day period was noted. At the end of the 15 days, the animals were killed and analysis of livers and femurs carried out as described earlier. These results are presented in Tables 3 and 4.

DISCUSSION

The results presented in this paper show that zinc in comparatively high concentrations causes a reduction in the weight and the fat content of rat livers. The reduction is marked in rats fed on a high-fat, low-protein diet and the results suggest that zinc possesses lipotropic activity.

Zinc brings about other changes also. On a stock diet, the 0.5% level of supplementary zinc oxide does not appear to lessen food intake, though it affects growth rate appreciably. On the stock ration, normal rats showed an increase in weight of 42 g. during an experimental period of 15 days. When, however, zinc oxide was added to the diet, the corresponding increases in weights were 37.5 and 13 g. for 0.5 and 1.0% levels of zinc oxide, respectively. The fresh weight of the kidneys also showed a corresponding decrease. At the 1% level zinc oxide caused a lowered food intake.

On a high-fat, low-protein diet, these changes were more pronounced. However, the decrease in the weight of the livers and their low fat content was particularly striking when the fat content of the diet was 10%. The content of fat in the diet thus appears to influence the changes brought about by the element.

Tables 2 and 4 show clearly that the development of bones is affected adversely in both the series. The observations of Smith & Larson (1946) that high-zinc diets bring about an anaemia, retarded growth rate and lowered food intake in rats, and the results presented in this paper show that zinc probably affects metabolism in general and assimilation of phosphorus in particular.

SUMMARY

1. The effect of supplements of zinc at levels of 0.5 and 1.0% on the livers and bones of rats has been investigated.

2. Zinc supplements caused a marked lowering of the fat content of livers of rats fed on a diet on which control rats developed fatty liver.

3. Zinc appears to act as a lipotropic factor in the concentrations studied. This element also interferes with development and mineralization of bones.

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REFERENCES

- Davidson, J. N. (1942). *Biochem. J.* **36**, 252.
 Day, H. G. (1942). *Fed. Proc.* **1**, 188.
 Day, H. G. & McCollum, E. V. (1940). *Proc. Soc. exp. Biol., N.Y.*, **45**, 282.
 Day, H. G. & Skidmore, B. E. (1947). *J. Nutrit.* **33**, 27.
 Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
 Gorter, F. J. (1939). *Ned.-ind. Bl. Diergeneesk.* **51**, 347.
 Grimmett, G. E. R., McIntosh, I. G., Wall, E. M. & Hopkirk, C. S. M. (1937). *N.Z. J. Agric.* **54**, 216.
 Holmberg, C. G. (1939). *Biochem. J.* **33**, 1901.
 Hove, E., Elvehjem, C. A. & Hart, E. B. (1937). *Amer. J. Physiol.* **119**, 768.
 Hove, E., Elvehjem, C. A. & Hart, E. B. (1940a). *J. biol. Chem.* **134**, 425.
 Hove, E., Elvehjem, C. A. & Hart, E. B. (1940b). *J. biol. Chem.* **136**, 425.
 Keilin, D. & Mann, T. (1939). *Nature, Lond.*, **144**, 442.
 Sadasivan, V. (1950). *Current Sci.* **19**, 10.
 Scott, D. A. (1934). *Biochem. J.* **28**, 1592.
 Scott, D. A. & Fisher, A. M. (1935). *Biochem. J.* **29**, 1048.
 Scott, D. A. & Fisher, A. M. (1938a). *J. clin. Investig.* **17**, 725.
 Scott, D. A. & Fisher, A. M. (1938b). *Amer. J. Physiol.* **121**, 253.
 Smith, S. E. & Larson, E. J. (1946). *J. biol. Chem.* **163**, 29.
 Stirn, F. E., Elvehjem, C. A. & Hart, E. B. (1935). *J. biol. Chem.* **109**, 347.
 Sutton, W. R. & Nelson, V. E. (1937). *Proc. Soc. exp. Biol., N.Y.*, **36**, 211.
 Todd, W. R., Elvehjem, C. A. & Hart, E. B. (1934). *Amer. J. Physiol.* **107**, 146.
 Wachtel, L. W., Hove, E., Elvehjem, C. A. & Hart, E. B. (1941). *J. biol. Chem.* **138**, 361.

In vitro Measurements of the Turnover Rate of Potassium in Brain and Retina

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It has been established by the isotope technique (Joseph, Cohn & Greenberg, 1939; Hahn, Hevesy & Rebbe, 1939; Fenn, Noonan, Mullins & Haage, 1941-2; Noonan, Fenn & Haage, 1941) that the potassium ions of animal tissues and of the body fluids continually interchange. The high concentration gradients of potassium between tissues and blood plasma, therefore, cannot be due to permeability barriers, but must be the resultant of two opposing processes: 'leakage' from the tissues, which tends to level out the concentration differences, and

'active', i.e. energy-fed, transport into the tissues, which counteracts the effects of 'leakage'. Under normal conditions the rates of the two opposing processes are usually equal and the problem presents itself of determining their velocities, i.e. the 'turnover rate' of potassium. Data published by Fenn *et al.* (1941-2) and Noonan *et al.* (1941) indicate great variations of the velocities from tissue to tissue. In general the visceral organs exchange potassium much more rapidly than skin, resting muscle or red blood cells. The only material for which accurate quanti-