during 24 and 48 hours, the level of latent enzyme released by pre-incubation with or without ribonuclease was 50 to 60% lower than in fed animals. The same phenomenon was observed with latent adenosinetriphosphatase[3]. Since fasting is known to decrease the RNA content of rat liver[9], a certain correlation might be established between the RNA content of mitochondria and the amount of latent enzymes. This is being investigated further.

Fig. 3 indicates that apparently the ribonuclease effect was not a non-specific protein effect. In fact, pre-treatment of mitochondria with albumin did not influence the rate of release of latent acid phosphatase. Fig. 3 shows also that the agglutination of mitochondria by addition of copper ions (the addition of $CuSO_4$ sucrose solution at a final concentration of $5.0 \cdot 10^{-4}$ *M* to mitochondrial suspension lowered the pH to 6.3 and the mitochondria agglutinated as with ribonuclease or Ca ions[11]) did not influence the release of latent enzyme.

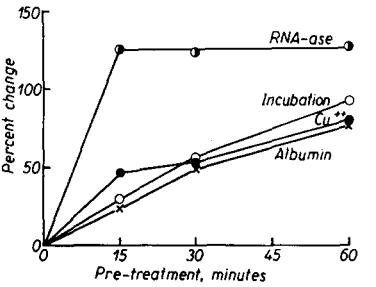

Fig. 3. Effect of various agents on latent acid phosphatase activity of rat liver mitochondria. 0.5 ml of a fresh 20% mitochondrial suspension in 0.25 *M* sucrose were pre-treated at 37° C during various periods of time with ribonuclease (1 mg), albumin (1 mg), Cu^{+2} ($5.0 \cdot 10^{-4}$ *M*, final concentration). The percent change in activity was calculated as in Fig. 1. For the Cu^{+2} experiment actual corrections were made for the slight activation of acid phosphatase activity by Cu^{+2}.

It remains to be proven that the ribonuclease effect on latent acid phosphatase is specifically due to the splitting of an inactive ribonucleoenzyme complex or to an unspecific damage caused by removal of ribonucleic acid from mitochondria. It is of interest in this respect that acid phosphatase of fresh liver microsomes—which is probably not present in latent form[1,2]—is not affected by ribonuclease[12].

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[1] J. BERTHET AND C. DE DUVE, *Biochem. J.*, 50 (1951) 174.
[2] J. BERTHET, F. BERTHET, F. APPELMANS AND C. DE DUVE, *Biochem. J.*, 50 (1951) 182.
[3] VAN R. POTTER, P. SIEKEVITZ AND H. C. SIMONSON, *J. Biol. Chem.*, 205 (1953) 893.
[4] W. C. SCHNEIDER, *J. Histochem. Cytochem.*, 1 (1953) 212.
[5] S. J. ROTH, *Nature*, 174 (1954) 129.
[6] M. R. MCDONALD AND B. P. KAUFMAN, *J. Histochem. Cytochem.*, 2 (1954) 387.
[7] W. C. SCHNEIDER AND G. H. HOGEBOOM, *J. Biol. Chem.*, 183 (1950) 123.
[8] C. ALLARD, G. DE LAMIRANDE, H. FARIA AND A. CANTERO, *Can. J. Biochem. and Physiol.*, 32 (1954) 383.
[9] M. F. HARRISON, *Biochem. J.*, 55 (1953) 204.
[10] G. DE LAMIRANDE, C. ALLARD AND A. CANTERO, *Science*, 12 (1954) 351.
[11] G. H. HOGEBOOM, W. C. SCHNEIDER AND M. J. STRIEBICH, *J. Biol. Chem.*, 196 (1952) 111.
[12] A. B. NOVIKOFF, J. RYAN AND E. PODBER, *J. Histochem. Cytochem.*, 2 (1954) 401.

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Heterogeneity of hypertensin preparations*

Several investigators have attempted the purification of hypertensin[1-9].

The purity of the various preparations reported varies widely and many discrepancies exist about the properties of the final product. For instance, KUETHER AND HANEY[8] and BUMPUS AND PAGE[7] have purified the hypertensin obtained from hog hypertensinogen and hog renin. The specific activity of KUETHER AND HANEY preparation is 24 times greater than BUMPUS AND PAGE product but on total hydrolysis gives one amino acid more (threonine) and no detectable N-terminal residue while BUMPUS AND PAGE report aspartic acid as N-terminal residue.

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The source of hypertensinogen and renin employed by different investigators is also very variable. Hog renin has been employed with ox[1,4], horse[2,5] and hog[6,8] hypertensinogen. PEART[9], has recently employed rabbit renin and ox serum. The number of different amino acids found in these hypertensins oscillates between 8 and 15.

During the purification of hypertensin prepared with hog renin and ox hypertensinogen, evidence of heterogeneity of the pressor activity was obtained by counter-current distribution in the systems 2-butanol, 0.1 *M* ammonium hydroxide and 2-butanol, 0.05 *M* sodium phosphate buffer pH 7.65.

These results led us to study the counter-current behavior of hypertensin preparations obtained with hypertensinogens from different animal species and hog renin.

The hypertensinogens were prepared by ammonium sulfate fractionation between 1.25 *M* and 2.20 *M* at pH 6.5, from hog and horse serum and ox plasma. The hypertensinase activity was destroyed by lowering the pH of the hypertensinogen solutions to 3.9 for 30 minutes at 25° C (Ref. 10, p. 133).

The hog renin used was prepared according to HAAS *et al.*[11] up to step 2, and then precipitated with ammonium sulfate at 0.65 saturation.

The hypertensin was obtained by mixing 1 volume of hypertensinogen with 0.15 volume of renin and incubating at pH 7.5 and 37° C for 20 minutes when the reaction was stopped with 3.5 volumes of 95% ethanol. The filtered alcoholic solution was evaporated to a small volume at room temperature and pH 5. The resulting aqueous solution was extracted with ether at pH 1.5, the excess ether was evaporated at room temperature and the remaining solution was saturated with sodium chloride and extracted twice with 1.5 volumes of 2-butanol. The combined butanolic extracts were evaporated to dryness below 40° C and the solid residue extracted with glacial acetic acid. The crude hypertensin obtained by evaporating to dryness the acetic acid was submitted to counter-current distribution without any further treatment. The results are given in Fig. 1 where diagrams *a*, *b* and *c* show that hog, horse and ox hypertensinogens each give rise to several active components on incubation with hog renin.

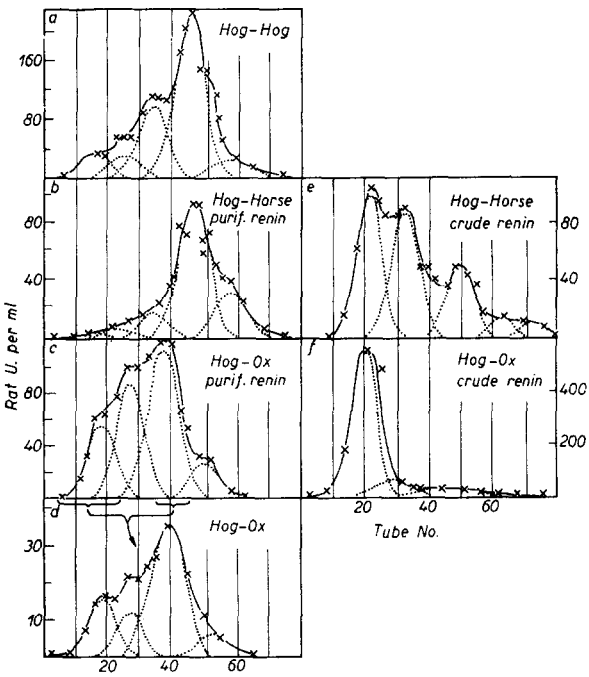

Fig. 1. Counter current distribution of different preparations of hypertensin. System: 2-butanol, 0.05 *M* sodium phosphate buffer, pH 7.65. No. of transfers: 80. Apparatus: 80 tubes, all glass machine with 2 ml capacity in each phase[13]. Temperature: 18° 20° C. Solid line: experimental curve. Dotted lines: theoretical distribution of a single substance[14]. Hog-Hog means: hog renin and hog hypertensinogen; Hog-Horse means: hog renin and horse hypertensinogen, and so on. The pressor activity was measured in nephrectomized rats anesthetized with nembutal[15]. One rat unit is the pressor activity of 0.2 ml of a standard hypertensin preparation. This amount raises the blood pressure 20 35 mm of mercury and is equivalent approximately to 0.01 Goldblatt unit[16].

To check the reliability of the fractionation procedure the contents of tubes 4 to 25 and 36 to 45 from run *c* were pooled and the pressor material therein was concentrated by extraction with 2-butanol and acetic acid as described before. Its counter-current distribution in the same system previously used gave diagram *d* essentially identical with *c*.

The importance of the purity of the renin in relation to the heterogeneity of the resulting hypertensin is demonstrated in diagrams *e* and *f*. The hypertensin used in these runs was prepared with hypertensinogens from horse and ox, as in *b* and *c* respectively, but the renin used was a less purified preparation obtained according to BRAUN-MENENDEZ *et al.* (Ref. 10, p. 343).

We also distributed the hypertensin obtained with horse hypertensinogen and purified hog renin, both previously dialyzed until the reaction for chloride ion was negative and the resulting diagram was similar although not identical to *b*.

The theoretical curves placed under each distribution curve point out the complexity of of the active material being analyzed. The actual number of different components can be much greater.

SKEGGS *et al.*[12] have found that the hypertensin obtained from horse hypertensinogen and hog renin, in a chloride-free medium (hypertensin I), can be converted to a different equally pressor compound (hypertensin II), apparently through the action of an enzyme in the plasma which requires halide or nitrate for activation.

PEART[9] has found two peaks of pressor activity in his preparations using partition chromatography. One of them had 5 % of the total activity.

It is difficult to explain at present the origin and physiological importance of the hypertensins reported here but it seems fairly well established that the preparations currently employed as starting materials for the purification may consist of a complex mixture of substances with similar biological activities. This fact may explain some of the discrepancies now existing in the field.

The origin of the different pressor activities, their pharmacology and connected problems are being investigated in this laboratory.

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1 E. BRAUN-MENENDEZ, J. C. FASCIOLO, L. F. LELOIR AND J. M. MUÑOZ, *J. Physiol.*, 98 (1940) 283.
2 P. EDMAN, *Arkiv Kemi Mineral. Geol.*, 22 A, No. 3 (1945) 1.
3 O. M. HELMER, *Proc. Soc. Exptl. Biol. Med.*, 74 (1950) 642.
4 L. C. CLARK, Jr., C. WINKLER, F. GOLLAN AND R. PHYLLIS FOX, *J. Biol. Chem.*, 206 (1954) 717.
5 L.T. SKEGGS, Jr., W.H. MARSH, J.R. KAHN AND N. P. SHUMWAY, *J. Exptl. Med.*, 100 (1954) 363.
6 F. M. BUMPUS, A. A. GREEN AND I. H. PAGE, *J. Biol. Chem.*, 210 (1954) 287.
7 F. M. BUMPUS AND I. H. PAGE, *Science*, 119 (1954) 849.
8 C. A. KUETHER AND M. E. HANEY, Jr., *Science*, 121 (1955) 65.
9 W. S. PEART, *Biochem. J.*, 60 (1955) vi.
10 E. BRAUN-MENENDEZ, J. C. FASCIOLO, L. F. LELOIR, J. M. MUNOZ AND A. C. TAQUINI, *Renal Hypertension*, Charles C. Thomas, Springfield, Illinois, 1946.
11 E. HAAS, H. LAMFROM AND H. GOLDBLATT, *Arch. Biochem. and Biophys.*, 42 (1953) 368.
12 L. T. SKEGGS, W. H. MARSH, J. R. KAHN AND N. P. SHUMWAY, *J. Exptl. Med.*, 99 (1954) 275.
13 P. H. BELL, personal communication.
14 L. C. CRAIG AND D. CRAIG, "Extraction and Distribution" in *Technique of Organic Chemistry*, Vol. III, edited by A. WEISSBERGER, Interscience Publishers, New York, 1950, p. 171.
15 E. BRAUN-MENENDEZ, unpublished work.
16 H. GOLDBLATT, H. LAMFROM AND E. HAAS, *Am. J. Physiol.*, 175 (1953) 75.

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Phosphoglycerate formation from pentose phosphate by extracts of *Thiobacillus denitrificans*

An enzyme system has been demonstrated in *Chlorella*[1] and spinach leaves[2] which carboxylates one molecule of pentose phosphate with the formation of two molecules of phosphoglycerate (PGA). This reaction is now considered to be the primary CO_2 fixing mechanism in photosynthesis[3,4]. When pentose monophosphate is the substrate, adenosinetriphosphate (ATP) is required for the formation of ribulose diphosphate[5], the actual CO_2 acceptor in the reaction[1,6,7].

Crude extracts of the chemosynthetic autotroph, *Thiobacillus denitrificans*, have been prepared which fix $^{14}CO_2$. Fixation was increased by the addition of ATP to the system and further increased by the addition of ribose-5-phosphate (R-5-P). The addition of R-5-P in the absence of ATP only slightly increased the amounts of $^{14}CO_2$ fixed. Table I shows the effect of adding R-5-P to the extract plus ATP. The mixture was deproteinized with trichloroacetic acid and a "phosphate ester" fraction prepared by treatment of the supernatant with barium acetate and ethanol at pH 8. Barium was removed from the precipitate with Dowex-50 (H form) and the solution so obtained freeze-dried. The product was chromatographed on Whatman No. 1