three samples is not a result of the presence of trypsin was demonstrated by chromatographing a mixture of trypsinogen Sample 1 and trypsin; the position of the trypsinogen peak was not altered.

In order to determine the chromatographic behavior of trypsinogen subjected to a minimum of chemical manipulations, fresh beef pancreas was extracted with 0.25 N H_2SO_4 in the manner described by KUNITZ AND NORTHROP[2]. The extract was dialyzed in the cold against 0.01 N HCl, first in a Visking 23–32 casing overnight and then in a 20–32 casing for 5 h, in an attempt to remove trypsin inhibitor. Apparently, however, inhibitor was still present, for the existence of trypsinogen in the dialyzed extract could not be demonstrated by the method described in Table I. The dialyzed extract was lyophilized, the dry powder was made up in about 1/10 the original volume of distilled water, and a portion of this solution was chromatographed. Activatable zymogen in an amount equivalent to 8 units/g fresh tissue (see Table I for the definition of a unit) was found in the effluent at the position assumed by trypsinogen Sample 1. Since the total amount of zymogen placed on the column could not be determined, it is not known whether all of the trypsinogen of the pancreas is of the type represented by Sample 1, or whether there is also present some of the varieties represented by Samples 2 and 3.

The chromatographic results, considered in conjunction with the data in Table I, demonstrate clearly that more than one trypsinogen can exist. If, as seems likely, only a single molecular species is elaborated by beef pancreas, the other trypsinogen(s) could only have arisen as a result of secondary changes, probably induced by enzymes. It appears that there may be a group of trypsinogens and trypsins analogous to the several chymotrypsinogens and chymotrypsins already known to exist[5,6].

The chromatographic conditions described in this communication are useful for the purification of trypsinogen of the type exemplified by Sample 1. Chymotrypsinogen, the most probable contaminant, is retarded much more by the column. The purification of trypsinogen Sample 1 is illustrated in Fig. 1, *b*. Activation of such purified trypsinogen should provide trypsin free from other proteolytic enzymes*.

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* The chromatographic purification of trypsinogen on IRC-50 has also recently been accomplished independently by P. J. KELLER and H. NEURATH (personal communication from Professor NEURATH).

Effect of chloramphenicol on protein and nucleic acid synthesis in isolated thymus nuclei*

Chloramphenicol has become an important tool in the study of the possible relationship of protein and nucleic acid syntheses. In some systems[1,2] chloramphenicol inhibits protein synthesis without affecting nucleic acid synthesis, while in others[3,4] both protein and nucleic acid syntheses are strongly inhibited. A completely different effect has been reported by ALLFREY *et al.*[5] in isolated calf-thymus nuclei where chloramphenicol (at a concentration of $3.2 \cdot 10^{-4} M$) failed to inhibit the incorporation of ^{14}C-amino acids into nuclear protein. As this effect suggests that protein synthesis in nuclei might differ somehow from that in other systems, we have investigated in more detail the effect of chloramphenicol on both ^{14}C-amino acid incorporation into protein and ^{32}P-orthophosphate incorporation into the nucleic acids of isolated calf-thymus nuclei.

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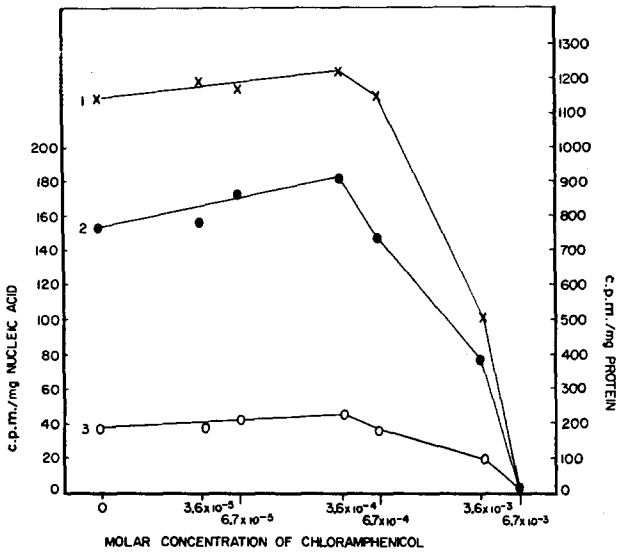

Fig. 1. Effect of chloramphenicol on ^{14}C-alanine incorporation into protein (curve 1) and ^{32}P-orthophosphate incorporation into RNA (curve 2) and DNA (curve 3).

Calf-thymus nuclei, prepared as described by ALLFREY *et al.*[5], were shaken for 90 min at 37° in a medium consisting of 1 ml nuclear suspension, 0.5 ml 0.1 *M* sodium phosphate–0.25 *M* sucrose buffer of pH 6.96, 0.4 ml 0.1*M* glucose containing 6.25 mg NaCl/ml, 0.1 ml ^{14}C-alanine (264,000 c.p.m.) or 0.1 ml ^{32}P-orthophosphate (270,000 c.p.m.), and chloramphenicol where indicated. The reactions were stopped by the addition of 10% $HClO_4$, the solution was diluted to 15 ml, and centrifuged at 1000 *g* for 15 min. The residue was washed 4 times with 5% $HClO_4$, once with ethanol, twice with an ether–ethanol–chloroform mixture (2:2:1), and finally with ether. The nucleic acids and protein were separated by the SCHMIDT-THANNHAUSER procedure[6]. The hydrolyzed ribonucleic and deoxyribonucleic acids were measured at 260 mμ in a Beckman spectrophotometer. Protein concentration was measured by the biuret reaction[7]. Aliquots of the hydrolyzed ribonucleic and deoxyribonucleic acids and the protein were dispersed evenly on glass planchets and assayed for radioactivity with a Nuclear Model D-47 gas-flow counter and standard scaling circuit.

Fig. 1 presents the effect of increasing concentrations of chloramphenicol on the incorporation of ^{14}C-alanine into nuclear protein. As chloramphenicol concentration is increased above that used by ALLFREY *et al.*[5], amino acid incorporation is progressively inhibited, and is completely inhibited at about $6.7 \cdot 10^{-3} M$. Furthermore, although the rates of incorporation of ^{32}P-orthophosphate into ribonucleic and deoxyribonucleic acids differ markedly, incorporation into both nucleic acids is inhibited in essentially the same manner as amino acid incorporation into protein. Chloramphenicol, therefore, apparently inhibits both protein and nucleic acid syntheses in isolated thymus nuclei in a manner similar to that already observed in ascites tumor cells[3] and in isolated ribonucleoprotein particles[4].

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