It was found that the excretion rate of the streptomycin administered together with alum was similar to that of streptomycin alone. The calculated recovery of the antibiotic in the urine was somewhat higher but not significantly so. Apparently the alum did not retard excretion. However, in all previous excretion studies in mice, the urine collected during the first hour or 2 following injection was a deep, reddish brown in color, very similar to that of the concentrated streptomycin solutions used. In the alum study, on the other hand, the urine was normal in color throughout the experiment, indicating that some of the pigmented impurities of the streptomycin preparation had been retained by the alum while the streptomycin was not.

Excretion of Streptomycin in Humans. Considerable data on the absorption and excretion of streptomycin in humans has already been reported (Table I). Consequently no special attempt was made to collect extensive data on this species. Limited data on streptomycin excretion have been collected on one adult and 3 infants to compare recovery rates in the urine with those found in mice, using the same bio-assay procedure.[10]

An adult patient, weighing 160 lb, was given 1,850,000 units of streptomycin subcutaneously in divided doses over a 6-day period. The average daily dose was 4,200 units per kg. The overall recovery of antibiotic in the urine was 75% of the streptomycin administered, but the ratio of units administered to units recovered in the urine varied somewhat during various stages of the study.

In 3 small children receiving 20,000 units of streptomycin per lb (ca 9100 units per kg) per day, intramuscularly, in interrupted doses or by continuous drip, the streptomycin administered which could be accounted for in the urine at any given period during the course of treatment again varied considerably with the individual. The overall recoveries of antibiotic in the urine were 31.5, 45.0 and 45.5% respectively. Although these are lower recoveries than some reported in adults, they fall within the general range found in man.

Conclusions. Whereas streptomycin is absorbed and excreted considerably faster in mice than in man, the percentage recovery of streptomycin in the urine of these 2 species is very similar. Hence, there is no reason to believe that this antibiotic is destroyed in mouse to any greater extent than in man.

Despite the fact that streptomycin is adsorbed on alkaline alumina, streptomycin administered in mixture with alkaline potassium aluminum sulfate is excreted as rapidly in mice as is streptomycin given alone.

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Proteins with Growth-Promoting Action on Tissue Cells *in vitro*.

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Among the many substances which contribute to the effectiveness of embryonic tissue extracts as growth-promoting media for cells *in vitro*, some have been found to be associated with the proteins of the extracts. The ease of inactivation of the extracts by heat and by proteolytic enzymes were early indications that proteins played some role in the effects which result in increasing the area of explanted tissue relative to the area of sister control-cultures.

The lability of the high molecular substances in the embryonic extracts makes it necessary to use only very mild methods in attempts to extract or purify active fractions. It has been found by Fischer and Astrup[1] that a rather high activity was associated with a fraction separated by the method used by Hammarsten[2] for preparation of nucleoproteins. Experiments have now confirmed

[1] Fischer, A., and Astrup, T., *Pflüg. Arch. ges. Physiol.*, 1943, **247**, 34.

[2] Hammarsten, E., *Hoppe-Seyl Z.*, 1920, **109**, 141.

that such fractions contain the relevant activity. It is, however, not yet certain that the activity necessarily resides in a protein, as distinct from a protein-complex containing nonprotein material, for one of the results of using mild preparative procedures is that labile, loose linkages may not be broken.

Examination of some crude nucleoprotein fractions has revealed no correlation between the activity and the proportions of the 2 types of nucleic acid, *i.e.* those containing ribose and desoxyribose. Activity has been found in fractions containing only a trace of desoxyribonucleic acid, so it is concluded that, if a nucleic acid is essential for activity, it must be one of the ribose and not of the desoxyribose type. Ribonucleic acid alone, in the depolymerised form in which it is isolated, does not appear to possess the activity. No method has so far been found for dissociating the ribonucleic acid from the protein, with which it is in firm combination, without denaturing the protein, so it cannot be stated whether the protein moiety alone shows any effect.

A convenient material for studying the active protein fraction of embryonic extracts has been prepared by precipitation of extracts in the cold (—15°C) by a modification of the method used by Hardy and Gardiner[3] and others for delipidation of serum and plasma proteins. Enbryonic extracts are made by mixing equal parts of tissue pulp and Ringer solution, and they therefore contain only material relatively easily extractable from the tissue. Such extracts, and hence also the powders prepared from them by cold-precipitation, are almost completely free from desoxyribonucleic acid. The stable, dry preparations of tissue protein obtained in this way give solutions in saline which possess the activity under discussion.

Examination of solutions of some of the powders so prepared in the Tiselius electrophoresis apparatus revealed that chick embryo preparations contain components of relatively few different mobilities. Preparations from calf embryos were even simpler in this respect, showing, in buffers of 2 different pH values, only a narrow range of mobilities. It was therefore thought to be of interest to examine the sedimentation of such a preparation in the ultracentrifuge; and through the courtesy of Dr. K. O. Pedersen of Upsala, this was done. For comparison with the calf embryo preparation, a powder prepared in exactly the same manner from the muscle of adult cow was also examined. The cow muscle preparation contained, as would be expected, several components, notably those with sedimentation constants (s_{20}) in the region of 1, 5 and 8. Electrophoretically this preparatoin was relatively homogeneous. The embryonic calf preparation, on the other hand, contained only one principal component, with $s_{20} = 4.8$, and corresponding therefore in size with the albumin group of proteins.

Of the low molecular substances precipitated with the proteins by the Hardy and Gardiner technic, a special examination of the phosphorus fractions has been made, since, as in plasma (Waymouth and Davidson[4]), a large proportion of inorganic and acid-soluble phosphorus appears in the final product. The greater part of these fractions could, however, be removed from the extract before precipitation, by dialysis overnight against running tapwater at about 10°C, without affecting the activity of the protein powder upon the growth of the tissue cultures.

These preliminary experiments have demonstrated that a type of growth-stimulating activity is associated with a protein fraction containing ribonucleoprotein, and possessing, for a material prepared by such a crude procedure, a surprisingly high degree of homogeneity in electrophoretic migration and in ultracentrifugal sedimentation. Growth-promoting activity was also found in adult tissue preparations made in a similar way, and it is thought that the type of substance responsible for the effect may be widely distributed in tissue cells, though masked in adult tissue extracts by the relatively higher proportion of nonactive components.

[3] Hardy, W. B., and Gardiner, S., *J. Physiol.*, 1910, **40**, lxviii.

[4] Waymouth, C., and Davidson, J. N., *Biochem. J.*, 1946, **40**, proc.