acids in all samples, indicates incorporation of radioactive acetate into blood fatty acids, regardless of the species. This is observed with human blood also (unpublished data). In all experiments except one, the radioactive count in digitonin cholesterol samples is higher than that in dibromo cholesterol samples, but is lower than that in digitonide mother liquor residues. This is in accordance with the observations of Schwenk *et al.*(5), who described the presence of cholesterol precursors in digitonin cholesterol samples and also in digitonide mother liquor residues.

It is very interesting to note that calf blood has a very active enzyme system for biosynthesis of cholesterol, while this activity is markedly decreased as the animal attains adulthood, as is evident from the results with cow blood. This pattern, however, is not observed with pig and piglet blood samples. We observed that a positive incorporation of acetate into cholesterol takes place in human myelogenous leukemic blood (unpublished data). Since leukemia is a disease of leukocytes, it was thought worthwhile to investigate the differential white cell pattern in blood of these species of animals (Table II). These observations, however, do not help in explaining the chemical findings, since total and the differential leukocyte counts of calf and pig blood samples, in which positive incorporation of acetate into cholesterol was observed, did not differ significantly or regularly from those of others. A very low radioactive count in cholesterol synthesized by rats, rabbits, piglets and cows as compared to complete lack of radioactivity in cholesterol in control experiments carried out with heat denatured cow and rat blood samples (Table I), indicates existence of the enzyme system responsible for biosynthesis of cholesterol from acetate in blood of these animals. Decisive incorporation of acetate into cholesterol in calf and pig blood samples strengthens the evidence in support of the existence of the enzyme system responsible for biosynthesis of cholesterol from acetate, in animal blood.

Summary. A study of biosynthesis of cholesterol *in vitro* from radioactive acetate in animal blood was undertaken. Degree of incorporation of acetate into cholesterol was higher in calf blood than in pig blood samples. Cholesterol synthesized in rat, rabbit, cow, and piglet blood samples had comparatively a very low radioactivity.

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Curarizing Effect of Neomycin and its Fractions in the Frog.* (24411)

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It is well known that drugs show multiplicity of action in clinical use. Neomycin, reported by Waksman(1) as effective antibiotic agent against streptomycin-resistant bacteria, can produce prolonged postoperative hypopnea or apnea when administered intraperitoneally in man(2-9). An attempt was made in this study to determine the mechanism of this respiratory depression and whether neomycin or its fractions A (Neamine), B and C possesses a curare-like action.

Methods. 67 adult frogs were employed. *In vivo* sciatic nerve-gastrocnemius muscle

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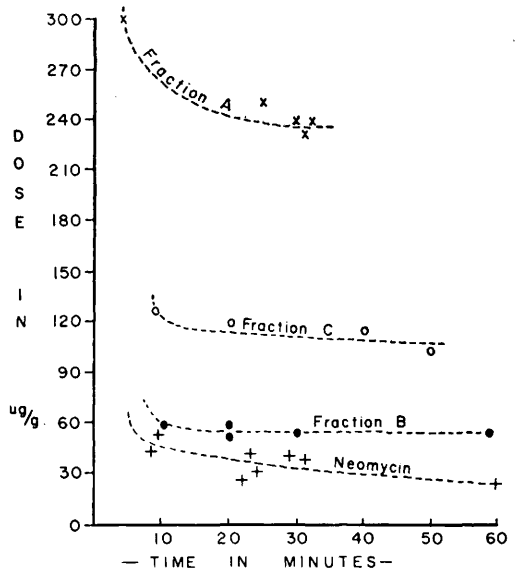

FIG. 1. The relationship of dose of Neomycin and its fractions in producing neuro-muscular blockade in the frog. Each symbol (× ○ ● +) represents 100% inhibition in time indicated.

preparation was prepared under ether or urethane anesthesia. Galvanic "make" and "break" stimuli, as well as tetanic stimuli with faradic induction coil, stimulated the moist neuromuscular preparations. Muscle contractions were recorded on revolving kymographic drum. Three control observations were made prior to introduction of the various substances into the dorsal lymph sac. Action of following substances was studied: (1) d-tubocurarine, in doses from 0.4 to 4 μg/g of body weight; (2) commercial neomycin, 20 to 120 μg/g; (3) neomycin fraction A (Neamine) 100 to 300 μg/g; (4) neomycin fraction B, 20 to 120 μg/g; (5) neomycin fraction C, 60 to 160 μg/g; (6) neostigmine 0.1 μg/g and edrophonium 2 μg/g of body weight. Commercial neomycin is a mixture of fractions B and C, where fraction B predominates. Current literature indicates that neamine has no antibiotic activity(10).

Results. A nerve-blocking curare-like action by neomycin, neomycin fractions A (Neamine), B and C was observed in every instance. A graded response was noted with neomycin or its fraction when minimal doses were used.

It was also observed that commercial neomycin and its fraction B possess the same potency on a mg/kg basis in producing neuromuscular blockade in the sciatic-gastrocnemius preparation. However, neomycin fraction C was at least half as potent than whole neomycin or its fraction B. Fraction A (Neamine) proved weakest of all (Fig. 1). An average dose of 270 μg/g was necessary to produce neuromuscular blockade in 10 minutes when neamine was used, in comparison with 140 μg/g of fraction C, 60 μg/g of fraction B, and 50 μg/g of commercial neomycin under same experimental conditions.

Neuromuscular blocking action of d-tubocurarine, neomycin and neomycin fractions B and C was effectively antagonized by neostigmine and edrophonium, while in the block produced by fraction A (Neamine), neostigmine and edrophonium did not restore neuromuscular transmission in doses used.

No appreciable difference in blockade by neomycin or its fractions B and C was observed when frogs were anesthetized with either ether or urethane.

Summary. Commercial neomycin and its fractions A, B and C possess a curarimimetic action as found when employing sciatic-gastrocnemius preparation of frogs. This action is more pronounced with commercial neomycin and fraction B than with fractions A (Neamine) and C. The blockade induced by neomycin, fraction B and C, but not by fraction A, was effectively antagonized with neostigmine or edrophonium in proper doses. Ether anesthesia did not potentiate this action in the frog when neomycin or its fractions B and C were used.

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Biosynthesis of Phosphoprotein in the Frog. (24412)

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The role of yolk proteins in development of amphibian embryo has been investigated by several workers(1,2). These studies have revealed the important role of phosphoproteins as a source of both amino acids and phosphorus for the growing organism. The isotopic and serological experiments of Flickinger and Rounds(3) carried out with the hen indicated that phosphoprotein is synthesized mainly in liver and is transported to the ovary by way of blood stream. It was therefore considered of interest to investigate whether a similar mechanism is operative in the case of the frog. The present investigation, carried out with the aid of radioactive phosphorus, provides evidence in favour of such an assumption, and demonstrates besides, the presence of a metabolically active phosphopeptide, utilization of which might represent an important step in biosynthesis of phosphoprotein.

Materials and methods. Two normal adult female frogs of similar size and age (*Rana esculenta*) were each given intraperitoneal injection of 100 μc of radioactive phosphorus as sterilized orthophosphate in isotonic saline at pH 7.0. This isotope was purchased from Radiochemical Centre, Amersham, U.K. One frog was killed at end of 6 hours, the other at end of 24 hours. Liver and ovary were removed immediately after sacrifice, and were homogenized in the cold with 10% trichloroacetic acid in a Potter-Elvehjem type of homogenizer. The various phosphorus-containing fragments of the tissues were separated according to procedure of Schneider(4) as modified by Friedkin and Lehninger(5). The acid-insoluble fraction, after exhaustive extraction for lipids, was dried and the phosphoprotein phosphorus estimated according to the method of Schmidt and Davidson(6). Phosphorus estimations in the various fractions were carried out according to the method of Fiske and Subbarow(7). For radioactivity measurements, suitable aliquots of the solution were evaporated to dryness in a planchet and were counted at infinite thinness in a thin end-window Geiger tube (window weight 2.1 mg/cm^2) coupled to a Panax counting equipment (Panax Equipment, Mitcham, Surrey, U.K.).

Results. The results of studies on distribution of radioactive phosphorus in the various fractions of liver and ovary of frog are given in Table I. The results indicate that in the phosphoprotein fraction of liver, there is a very high rate of renewal of phosphorus. This observation is similar to the finding of earlier workers(8,9). Specific activity of this fraction is 4-5 times higher than that of phospholipid fraction. The results also show that rates of renewal of phosphorus in the various fractions of ovary are generally very low.

Analysis of acid-soluble organic phosphates of liver. The high activity of the acid-soluble organic phosphates of liver led to a detailed characterization and analysis of this fraction for the possible presence of any active component that might be involved in biosynthesis of phosphoproteins. For characterization of this fraction, the organic phosphates were precipitated as barium salts as described(10). The free phosphate esters, after removal of barium, were subjected to paper strip electrophoresis in barbital-barbiturate buffer (pH

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