cellular distribution pattern of kallikrein in the various species studied is very similar to that in the guinea-pig; the kallikrein in all these species is held in dense granules since the results indicate that the major part of particulate kallikrein sediments at low *g*.

Although in the previous study kallikrein granules from the guinea-pig submaxillary gland were clearly differentiated from mitochondria and lysosomes,[2] no such comparison has been made in the cat, rabbit, dog and rat. However, experiments are in progress in which subcellular particles in the mitochondrial fraction ($14{\cdot}25 \times 10^4$; P_2) of submaxillary gland homogenates of these species have been centrifuged on sucrose density-gradients and the isopynic equilibrium point for kallikrein granules compared with that of mitochondria and lysosomes.

The physiological function of submaxillary kallikrein is not established. The experimental evidence indicates that kallikrein does not regulate functional vasodilatation in the guinea-pig[5, 6] and rabbit.[7] Since the present results indicate that the subcellular distribution of the kallikrein granules in the various species studied is identical to that in the guinea-pig and rabbit it may be assumed that in the cat, dog and rat also, kallikrein is not involved in functional vasodilatation.

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The isolation of an ACh-binding fraction from ox diaphragm muscle

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VARIOUS unsuccessful attempts have been made to isolate and identify acetylcholine (ACh) receptor-like substances. Although Chagas, Ehrenpreis and Takagi have reported attempts to isolate and identify the ACh receptor, their experimental results are not yet definitive.[1–3]

It has been observed that glycerinated striated muscles do not respond to ACh, in contrast to normal muscles, but that after glycerination they respond to adenosine triphosphate (ATP).[4] Since Waser has shown that there is a rich supply of ACh receptors in diaphragm muscles,[5] the effect of glycerol treatment on the response to ACh ($3{\cdot}08 \times 10^{-5}$ M) or ATP ($6{\cdot}6 \times 10^{-3}$ M) was examined in rat diaphragm muscle denervated 5–17 days previously in order to make it sensitive to exogenous ACh. A typical example of the results is shown in Fig. 1.

It was clearly observed that the responses to ACh of muscles treated with 10% glycerol had disappeared completely, while the responses to ATP began to appear gradually with increasing of glycerol concentration. The response to KCl of muscles untreated with glycerol was used as a control (100 per cent contraction).

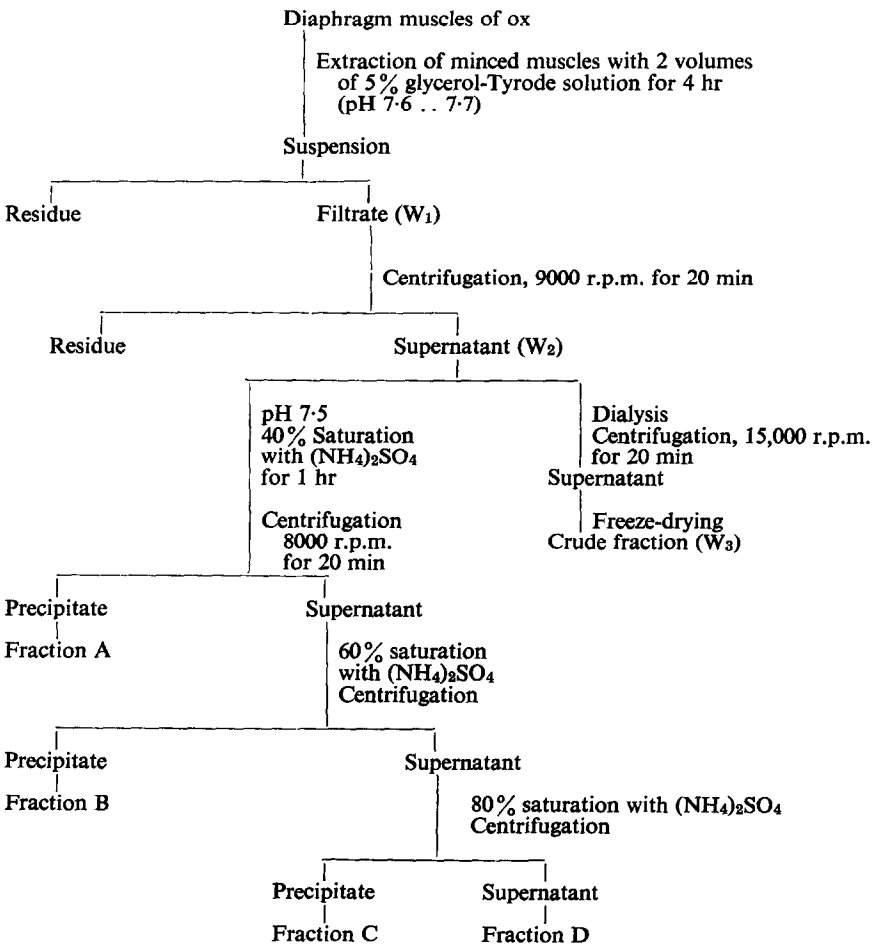
Subsequently it was studied whether an ACh-binding fraction could be extracted from the diaphragm muscle of the ox with 5% glycerol solution. The extraction procedure is shown in Table 1 In order to study the ACh-binding properties of the fraction prepared by dialysis and freeze-drying, the "back-reaction" caused by changing the agonist–receptor equilibrium in isolated organ experiments according to Magnus method was used. The possible mechanism of the "back-reaction" is as follows:

$$\begin{array}{ccc} (A) + (R) & \rightleftharpoons & (AR) \\ + & & \\ (R') + (B) & \rightleftharpoons & (BR') \\ \Updownarrow & & \\ (AR') & & \end{array}$$

The contraction of muscle segment by ACh (A) is dependent on the interaction of ACh with the specific receptor (R). The contraction is inhibited by the administration of a fraction (R′) which binds ACh (back-reaction), then it should be possible to antagonize the back reaction by the competitive antagonist of ACh (A), *d*-tubocurarine (B).

Rabbit's tracheal muscles were used as experimental organs since they do not show a reaction to *d*-tubocurarine (*d*-TC) itself. It was found that the crude fraction W_3 and the final fractions A, B, C and D (see Table 1) all caused a back reaction. However, the back reactions caused by the fraction

TABLE 1. THE EXTRACTION PROCEDURE FOR ACh-BINDING SUBSTANCES



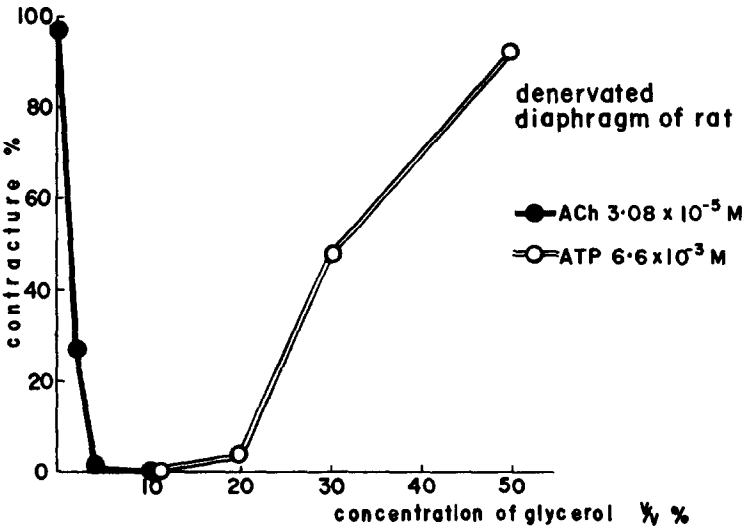


FIG. 1. The relationship between the glycerol concentration and the percentage of ACh or ATP contracture relative to the maximum response of 0·1M K_2SO_4, using chronically denervated diaphragm muscles of rats.

W_3, A, B and C were reduced or almost abolished in presence of neostigmine (5×10^{-6} g/ml), whereas the back reaction caused by fraction D was not reduced. It was concluded that the back reaction observed with the fractions W_3, A, B and C might be caused by the fact that fractions were rich in ACh-esterase, but that fraction D contained another ACh-binding substance.

The observations that the response to ACh (5×10^{-7} g/ml) was reduced by fraction D (0·5%) and then again restored by *d*-TC ($2{\cdot}5 \times 10^{-5}$ g/ml) and also that fraction D did not cause a back

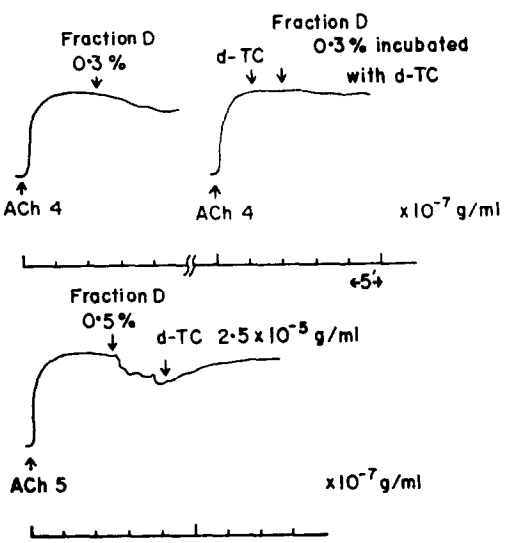


FIG. 2. The "back reaction" of ACh responses by fraction D and the restoration of its reaction by *d*-TC, using rabbit's tracheal muscles to assay ACh.

reaction if *d*-TC was added before (see Fig. 2) are in agreement with the suppositions made above The back reaction caused by the fraction W_3, A, B and C were not antagonized by *d*-TC.

Fraction D was further fractionated by gel filtration on Sephadex G-75 with 0·05 M sodium phosphate buffer (pH 6·6). The size of the column was 2·0 cm × 38·2 cm and the flow rate was about 15 ml/hr. Finally, fraction D was separated into fraction I (1·6 per cent of total), fraction II (0·7 per cent) and fraction III (97·7 per cent).

These three fractions were subjected to agar gel electrophoresis (13·5 V/cm at 5° for 15 min. and on a 1·5-mm thick layer of 1·5 per cent agar gel in Tris-EDTA borate buffer). This fraction D contained about 98 per cent of a pure single substance.

It was observed that fraction D (in 0·05 M sodium phosphate buffer, pH 6·6) had absorption maxima at 280, 409, 500 and 630 mμ, with the strongest absorption at 409 mμ. This pattern corresponds to haem, suggesting that fraction D might, mainly, consist of myoglobin.[6] To provide further support for this suggestion, fraction D was fractionated by IEC on DEAE-cellulose with Tris-HCl-buffer (pH 8·4) with a step-wise change in the ionic strength.[7] It was observed that ferrimyoglobin was eluted first, and this was followed by oxymyoglobin.

In view of the above results it is concluded that the ACh-binding fraction isolated from ox diaphragm muscle mainly consisted of myoglobin.

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Study on the stability of lysosome membranes—IV. Protection of liver lysosomes from the labilizing effect of chlorpromazine with succinate and glutamate *in vivo*

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SMALL doses of chlorpromazine (CPZ) stabilize and large ones labilize lysosomes [1]ATP and riboflavine protect these particles from the injuring action of the drug [2, 3] It is possible that the stabilizing effect of ATP is due to the protection of succindehydrogenase (SD) This interpretation is in agreement with the hypothesis according to which *in vivo* CPZ labilizes lysosome membranes indirectly, by causing primary disturbances in a bioenergetic pathway [2–4] ATP may protect SD from the inhibitory