TABLE 1. EFFECT OF SALICYLATE (10^{-3} M) ON THE INCORPORATION OF ^{14}C-ACETATE INTO CHOLESTEROL AND FATTY ACIDS AND ^{14}C-MEVALONATE INTO CHOLESTEROL

Substrate	No.	Condition	Unsaponified fraction (sterols) (cpm/g of liver)	(% inhibition)	Saponified fraction (fatty acids) (cpm/g of liver)	(% inhibition)
1-^{14}C-acetate	1	control	12,757	—	2699	—
		salicylate	9425	26	1634	40
	2	control	18,931	—	4073	—
		salicylate	12,412	34	1678	59
	3	control	33,212	—	6135	—
		salicylate	23,022	30	3080	50
2-^{14}C-mevalonate	1	control	4200	no inhibition		
		salicylate	4408			
	2	control	4299			
		salicylate	4979			
	3	control	4732			
		salicylate	4367			

in the incorporation of acetate into the fatty acids fraction was a little more marked and reached an average of 50 per cent (the same percentage was observed by P. Goldman in his experiments).[5] Meanwhile the salicylate had no effect on the incorporation of mevalonate into cholesterol. It must therefore act on the earlier stages of biosynthesis of cholesterol, connected with conversion of acetate. The data obtained in the present work does not contradict the suggestion that salicylate inhibits the reaction catalysing the carboxylation of acetyl CoA. The lowering of serum cholesterol by salicylate[2] may be connected with the inhibition of its biosynthesis.

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Comparative study of the subcellular distribution of submaxillary kallikrein

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SUBCELLULAR studies indicate that submaxillary kallikrein is held in granules. On differential centrifugation the kallikrein-containing particles sediment at relatively low g^1 and on density-gradient

centrifugation reach their isopynic position between 1·7 M and 1·8 M sucrose.[2] So far the kallikrein-containing granules in the submaxillary gland of the guinea-pig[1, 2] and the rat[3] have been studied. Since the intracellular localisation of kallikrein in granules probably relates to its function it was important to determine whether submaxillary kallikrein in other species is similarly held in particles.

Submaxillary glands of the guinea-pig, cat, rabbit, dog and rat were homogenised in 0·32 M sucrose. The method of gland removal and homogenisation was similar to that described previously.[2] The procedure for differential centrifugation involved separation of the nuclear fraction on a B.T.L. centrifuge and subsequent fractionation on an M.S.E. 40 centrifuge. The *g* min used for the separation of particles was as follows: nuclear fraction, $8{\cdot}25 \times 10^2$ (P_1); mitochondrial fraction, $14{\cdot}25 \times 10^4$ (P_2); lysosomal or light mitochondrial fraction, $6{\cdot}15 \times 10^5$ (P_3) and microsomal fraction $8{\cdot}7 \times 10^6$ (P_4). Kallikrein was measured by its ability to release kallidin from heated, dialysed dog plasma; the released kallidin was directly assayed on the isolated guinea-pig ileum in the presence of the kininase inhibitor, L-cysteine.[2] The biological method for measuring kallikrein was preferred because the chemical assay using synthetic substrates lacked specificity due to the presence of other tissue esterases in the homogenates; at least five separate enzymes capable of hydrolysing these synthetic substrates have been demonstrated in homogenates of rat submaxillary gland.[4]

Since in the present experiments the intracellular distribution pattern for guinea-pig submaxillary kallikrein was similar to that of a previous comprehensive study[2] (Table 1), the findings in the guinea-pig were compared with those obtained in the cat, rabbit, dog and rat. Table 2 shows that the sub-

TABLE 1. SUBCELLULAR DISTRIBUTION OF KALLIKREIN IN THE SUBMAXILLARY GLAND OF THE GUINEA-PIG

		A	B	C
Sucrose molarity		0·32M	0·7M	0·32M
Fractions	*g* min			
P_1 Nuclear	$8{\cdot}25 \times 10^2$	—	—	24·6
	$6{\cdot}5 \times 10^3$	—	32·7	—
	$10{\cdot}99 \times 10^3$	46·1	—	—
P_2 Mitochondrial	$9{\cdot}05 \times 10^4$	—	31·4	—
	$14{\cdot}25 \times 10^4$	—	—	35·1
P_3 Lysosomal	$6{\cdot}15 \times 10^5$	17·2	7·6	5·5
P_4 Microsomal	$8{\cdot}7 \times 10^6$	—	2·6	1·1
	$9{\cdot}6 \times 10^6$	0	—	—
S Supernatant		36·8	25·3	33·2
Total recovery		179·5%	99·2%	106·9%
No. of experiments		4	4	3

The activity of kallikrein in each fraction is expressed as a percentage of total recovery.
A: [1]Bhoola & Ogle, 1966 B: [2]Bhoola 1968 C: Present results

TABLE 2. COMPARISON OF THE SUBCELLULAR DISTRIBUTION OF KALLIKREIN IN SUBMAXILLARY GLANDS OF VARIOUS SPECIES

Fractions	*g* min	Guinea-pig	Cat	Rabbit	Dog	Rat
P_1	$8{\cdot}25 \times 10^2$	24·6	9·9	16·7	10·6	5·0
P_2	$14{\cdot}25 \times 10^4$	35·1	36·9	29·0	37·3	38·7
P_3	$6{\cdot}15 \times 10^5$	5·5	8·2	5·2	3·6	1·5
P_4	$8{\cdot}7 \times 10^6$	1·1	3·4	4·8	3·8	0·6
S		33·2	47·1	44·2	44·5	55·7
Total recovery		106·9%	68·4%	90·8%	75·0%	77·7%
No of experiments		3	3	2	2	2

The activity of kallikrein in each fraction is expressed as a percentage of total recovery.

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).