and were killed the following day. Specimens of the prostate, spleen, liver, lymph nodes, bone marrow, skin and subcutaneous tissue, adrenal glands, and, when possible, the testes were fixed in Heidenhain's "susa" fluid. Sections were cut 10 microns thick and stained with dilute carbol-fuchsin or weak eosin. The activity of the reticulo-endothelial system was measured by the number of dye-bearing cells, and the intensity of the vital staining in the organs and tissues was examined.

Results

The results showed that in both the intact and the orchidectomized animals oestradiol benzoate produced hyperplasia and stimulation of the reticulo-endothelial macrophages, mainly in the spleen (Special Plate, Figs. 1, 2, and 3), liver, lymph nodes, and prostate, and that the degree of stimulation was roughly proportionate to the duration of the hormone treatment. In the orchidectomized animals the reticulo-endothelial system was more active, and oestradiol benzoate produced a greater response than in the intact animals.

In the spleen, prolonged oestrogenic stimulation appeared not only to produce a greater formation and activation of the reticulo-endothelial cells but also to assist their mobilization and liberation into the circulation. This was noted especially in the spleen of orchidectomized animals given the hormone for four weeks, in which the splenic sinuses were dilated and contained, in some animals, numerous dye-bearing cells, and in others a moderate number. In others the sinuses were practically empty (Fig. 4), although in the same animals the splenic vein and the intrahepatic branches of the portal vein contained a great number of dye-bearing cells. Furthermore, the peritoneal mesothelial cells covering the spleen became columnar and showed dye granules in the infranuclear zone as a result of the oestrogenic treatment, which confirms the findings recorded by Helmy and Nicol (1951) in female animals.

In the prostate, the dye-bearing cells appeared in the loose areolar tissue between the acini (Fig. 5). The vitally stained cells were very few in the intact animals not given hormone, but became numerous after oestrogenic treatment, and still more numerous when this was combined with orchidectomy. The thin layer of fibrous tissue which normally surrounds the prostatic acini became progressively thicker with hormone treatment, and the prostate showed the greatest degree of fibrosis in the orchidectomized animals which received oestradiol benzoate for four weeks (Fig. 6). Fibrosis was particularly marked in the region of the utriculus musculus. The arrangement of cells around the prostatic acini in the hormone-treated animals suggests that oestradiol benzoate leads to the appearance of macrophages around the acini and that if the treatment is maintained these macrophages change into fibroblasts and fibrous tissue. Orchidectomy also seems to influence both the deposition of the macrophages and their transformation into fibroblasts. The macrophages in the prostate probably come mainly via the blood stream from the spleen and other reticulo-endothelial organs, where their mobilization is stimulated by the oestrogens, and partly from proliferation of pre-existing local cells having mesenchymal potencies.

Conclusions

The stimulating effect of orchidectomy on the reticulo-endothelial system may be explained by: (1) The absence of the neutralizing effect of androgens on oestrogens which are normally found in the male animal and are probably produced by the suprarenal glands. This neutralizing effect was shown by Ihrke and D'Amour (1931), Browman (1937), and Robson (1938). (2) The stimulating effect of orchidectomy on the anterior lobe of the pituitary, leading to the output of more adrenotropic hormone, which in turn leads to the production of more oestrogens by the suprarenals.

The results of the present investigations, therefore, show that oestrogens and orchidectomy both stimulate the reticulo-endothelial system and in this manner probably increase the

general resistance against cancer; that in the prostate they induce the appearance of macrophages and later of fibrous tissue, which acts as a local defence against the spread of cancer; and that oestrogenic treatment combined with orchidectomy produces a more intense result than either alone.

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RESPONSE IN DOGS TO RELAXANTS DERIVED FROM SUCCINIC ACID AND CHOLINE

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It has recently been reported (Hall, 1952) that suxethonium is a useful relaxant in operations on dogs, and as such is a welcome addition to the surgical practice concerned with this species. There was, however, a difference between the effects of the drug in dogs and in man, dogs being more sensitive and responding with longer apnoea to smaller doses. In an investigation on succinylcholine sensitivity in man (Evans, Gray, Lehmann, and Silk, 1952) a correlation had been found between low pseudo-cholinesterase level in the plasma and prolonged response to succinylcholine. This observation, and experiments *in vitro*, suggested that in man the drugs produced depolarization, not only directly but also by inhibiting the true cholinesterase, thus raising the acetylcholine concentration in the neuromuscular end-plates, and that the short duration of this effect was due to the rapid hydrolysis of succinylcholine by the pseudo-cholinesterase of the plasma. We therefore decided to investigate the enhanced effect of suxethonium in dogs along these lines by finding out whether there were differences between the enzyme systems of man and dog.

Methods

All observations in dogs except one were made in the course of necessary treatment. "Brevdil E," which used to be suxethonium di-iodide and is now obtainable commercially as the dibromide, and "scoline" (suxamethonium chloride), which is succinylcholine dichloride, were both used *in vivo* and *in vitro*. There was no major difference in their behaviour. All doses used *in vivo* and *in vitro* are expressed in terms of the active cation. The *in vitro* techniques followed were the same as described previously (Evans *et al.*, 1952). They depend on the evolution of CO₂ from bicarbonate buffer by the products of ester hydrolysis; the activity

of the enzymes is expressed as $\mu\text{l.}$ of CO_2 formed per minute by 1 ml. of serum or packed red cells at 37°C. (see McArdle, 1940).

Results

In 25 operations on dogs suxethonium was given in dosages mostly from 0.08 to 0.15 mg. per lb. body weight; in some instances the amounts administered were even less. To man four to six times these dosages are administered, yet the apnoea produced in dogs was longer than that seen normally in man. It has to be conceded that in the chest conditions hyperventilation may have played a part in extending the duration of apnoea.

We then established that in dogs, as in man, succinylcholine and suxethonium inhibit the hydrolysis of acetylcholine both by the true cholinesterase prepared from red

response to succinylcholine. However, there was a considerable difference between the level of true cholinesterase of the red cells in man and dog. The values for man are about 80 to 120 (although very occasionally they may be as low as 60 to 80); and it will be seen in Table II that we found them to be six to eight times lower in dogs.

As pointed out in the introduction to this paper, the action of succinylcholine in man can be visualized as a combination of it with the two cholinesterases. True cholinesterase + succinylcholine causes the pharmacological effect, and pseudo-cholinesterase + succinylcholine brings it to its rapid end. The duration of apnoea would then depend on the dosage of succinylcholine given and on the concentration of the two esterases combining with it—that is, equilibria will be established between the free succinylcholine and the succinylcholine adsorbed both on the true

TABLE I.—*Succinylcholine and Suxethonium in Dogs' Blood. Breakdown by Pseudo-cholinesterase, Inhibition of Acetylcholine Breakdown by Both Pseudo-cholinesterase and True Cholinesterase*

Substrate (Active Cation)	$\mu\text{l. CO}_2$ Liberated by 1 ml. Serum or Packed Red Cells per Minute at 37°C.				
	Acetylcholine	Succinylcholine	Suxethonium	Acetylcholine + Succinylcholine	Acetylcholine + Suxethonium
Pseudo-cholinesterase of serum:					
Acetylcholine:					
6.5 mg./ml.	68	2	—	14	—
Succinylcholine:					
13 mg./ml.	43	4	—	20	—
Suxethonium:					
14 mg./ml.	45	7	2	17	8
True cholinesterase of red cells:					
Acetylcholine:					
0.65 mg./ml.	18	—	—	1	—
Succinylcholine:					
1.3 mg./ml.	16	—	—	2	—
Suxethonium:					
1.4 mg./ml.	18	—	—	2	0

cells and by the pseudo-cholinesterase of the plasma, and, furthermore, that succinylcholine and suxethonium themselves are hydrolysed by pseudo-cholinesterase and not by the red-cell enzyme (for details see Table I). The inhibition of acetylcholine breakdown by dog's serum in the presence of succinylcholine had already been shown by Bovet and his colleagues (1951).

In man the cause of prolonged apnoea after succinylcholine had been a low activity of the pseudo-cholinesterase of the plasma. We therefore thought at first that dogs might normally have a much lower pseudo-cholinesterase in their plasma than man. Table II shows that, although the values for pseudo-cholinesterase tend to be lower in dog than in man, in whom the normal range is 55 to 120 units, only one dog had an esterase value below 40, which might in man cause a significantly prolonged apnoeic

TABLE II.—*Levels of Pseudo-cholinesterase (Serum) and True Cholinesterases (Red Cells) in Dogs' Blood. None of the Dogs Suffered from Anaemia or Liver Disease*

Animal	$\mu\text{l. CO}_2$ Liberated per ml. Serum or Packed Red Cells per Minute at 37°C.	
	Pseudo-cholinesterase (6.5 mg. Acetylcholine/ml.)	True Cholinesterase (0.65 mg. Acetylcholine/ml.)
Greyhound	66	15
Mongrel	66	10
Mongrel	86	9
Cairn	55	14
Greyhound	68	18
Dachshund	49	9
Alsatian	65	13
Puppy	50	20
Mongrel	43	16
Mongrel	75	16
Labrador	33	21
Labrador	49	19

cholinesterase on the one hand and on the pseudo-cholinesterase on the other. Of these the combination pseudo-cholinesterase + succinylcholine is unstable and results in hydrolysis of the adsorbed drug. This will free pseudo-cholinesterase, which will thus become available for the remaining free succinylcholine. As the free drug is also in equilibrium with that combined with the true cholinesterase a fall in its concentration will result in a release of succinylcholine from that combination and the pharmacological effect will progressively abate.

The difference between the action of succinylcholine or suxethonium in man and dog can be explained on the basis of these equilibria. With a low concentration of true cholinesterase less free succinylcholine or suxethonium is required for the inhibitory combination which produces subsequent depolarization, and, although the destruction by pseudo-cholinesterase is almost as efficient as in man, the drain on the remaining free choline derivative cannot have the same effect because in the dog lower concentrations would still suffice to occupy the smaller amount of true

TABLE III

Type of Dog and Sex:	Mongrel, Male	Mongrel, Female	Labrador, Male	Labrador, Male
Age and weight	2 years; 25 lb. (11.3 kg.)	12 years; 22½ lb. (10.2 kg.)	6 months; 41 lb. (18.6 kg.)	3½ years; 63 lb. (28.6 kg.)
Premedication	Pethidine, 25 mg.; scopopolamine, 1/200 gr.	Pethidine, 15 mg.; scopopolamine, 1/200 gr.	Pethidine, 50 mg.; scopopolamine, 1/200 gr.	Pethidine, 200 mg.; scopopolamine, 1/100 gr.
Thiopentone (g.)	0.3	0.3	0.45	0.6
Maintenance	$\text{N}_2\text{O/O}_2$, 50:50	$\text{N}_2\text{O/O}_2$, 50:50	$\text{N}_2\text{O/O}_2$, 50:50; thiopentone, q.s.	$\text{N}_2\text{O/O}_2$, 50:50; minimal tritlene; thiopentone, q.s.
Pseudo-cholinesterase in plasma (units)	43	75	33	49
True cholinesterase in red cells (units)	16	16	21	19
Succinylcholine (mg.) (active cation)	2.5	2.2	5	7
Duration of apnoea (min.)	14	12	15	13
Thiopentone (g.)	0.05	—	—	—
Succinylcholine (mg.)	2.5	2.2	—	—
Duration of apnoea (min.)	18	13	—	—
Pseudo-cholinesterase injected (units)	21,000	21,000	21,000	21,000
in plasma (units)	102	111	54	63
True cholinesterase in red cells (units)	—	15	18	16
Thiopentone (g.)	0.1	0.05	0.05	0.1
Succinylcholine (mg.)	2.5	2.2	5	7
Duration of apnoea (min.)	4	No apnoea*	4.5	No apnoea*
Succinylcholine (mg.)	—	—	5	—
Duration of apnoea (min.)	—	—	5	—

* Though there was no apnoea there was shallow breathing, needing some assisted respiration in dog 2.

cholinesterase available. However, it should be possible to increase the pseudo-cholinesterase of the plasma in the dog and thus to lower the concentration of free succinylcholine or suxethonium so rapidly that even the low concentration of the drug required for inhibition of the true cholinesterase in that animal will be effectively attacked. We therefore injected a highly concentrated solution of pseudo-cholinesterase intravenously into four dogs in the course of operations for mastectomy or conditions requiring orthopaedic treatment, and compared the effect of equal amounts of suxethonium given before and after the pseudo-cholinesterase level of the plasma had been increased.

As will be seen in Table III, after the pseudo-cholinesterase had been effectively raised succinylcholine was as short-acting in dog as it is known to be in man. To exclude the effects of premedication and the possibility that apnoea was due to artificial hyperventilation, one experiment was performed in which only thiopentone was used for induction and no artificial respiration was given during apnoea beyond one inflation per minute. Table IV shows that the

TABLE IV.—*Adult Mongrel Bitch, Weight 31 lb. (14 kg.)*

0 min.	0.5 g. thiopentone	Recording apparatus for blood pressure and respiratory rate connected
18 "	0.1 g. thiopentone	Pseudo-cholinesterase, 46; true cholinesterase, 18
23 "	3.5 mg. suxethonium	16 min. apnoea
43 "	0.1 g. thiopentone	
48 "	3.5 mg. suxethonium	11 min. apnoea
70 "	2 mg. succinylcholine	5 min. apnoea
83 "	0.1 g. thiopentone	
93 "	injection of 21,000 units pseudo-cholinesterase	
97 "	Pseudo-cholinesterase, 76; true cholinesterase, 15.5	
102 "	3.5 mg. suxethonium	no apnoea, 3 min. respiratory depression
112 "	3.5 mg. suxethonium	no apnoea, 4 min. respiratory depression
122 "	2 mg. succinylcholine	no apnoea, 2 min. respiratory depression
130 "		Pseudo-cholinesterase, 78; true cholinesterase, 15

raising of the pseudo-cholinesterase level was effective under these conditions. Blood-pressure readings were obtained throughout and allowed an exclusion of asphyxia. Both brevidil E and scoline were used and noted as such in terms of active cation.

Discussion

Our findings support the suggestion that the effect of succinylcholine and suxethonium is the outcome of an interplay between these compounds and the two cholinesterases. The fact that the dog is much more sensitive than man would then be explained by the finding of a six to eight times lower true cholinesterase concentration in the red cells. Similarly, the fact that the sensitivity of the dog can be lowered to levels seen in man by raising the pseudo-cholinesterase level of the plasma can be understood on the bases of these considerations. We have assumed that the two esterases in man and dog are the same enzymes, and that the differences in activity of blood and plasma are due to different enzyme concentrations. It is, of course, possible that there are species variations and that the affinities rather than the concentrations are different. However, the interplay between compounds and enzymes would have the same outcome whether the enzymes differ in concentration or in affinity. Apart from theories, our findings have two practical consequences: the absence of side-reactions in the dog when given human cholinesterase preparations justifies its trial in man in cases of succinylcholine sensitivity. The use of the enzyme will be of advantage in veterinary medicine, as it will convert succinylcholine and suxethonium from comparatively long-time relaxants into compounds acting for a few minutes only, thus bringing into the surgery of the dog the advantages found in the use of succinylcholine and suxethonium in man.

Summary

Dogs are more sensitive to suxethonium and succinylcholine than man. When injected with doses about one-fifth of those used in man they react with an apnoea of about five times longer duration.

The pseudo-cholinesterase concentration in dogs' serum is not much lower than that in man, but the true-cholinesterase concentration in the red cells is only about one-seventh. It is suggested that there is a correlation between higher sensitivity and lower true cholinesterase and that raising or lowering any of the two enzymes will raise or lower the time of apnoea after succinylcholine or suxethonium.

In five dogs raising of the pseudo-cholinesterase level in the circulating plasma shortened effectively the time of apnoea after suxethonium or succinylcholine. The theoretical and practical significance of these findings is discussed.

We should like to express our thanks to Dr. E. B. McLean, of the U.S.A., who provided us with a pseudo-cholinesterase preparation containing 21,000 units per ml. This solution will in due course become available commercially under the name of "cholasen."

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EFFECT OF PSEUDO-CHOLINESTERASE LEVEL ON ACTION OF SUCCINYL- CHOLINE IN MAN

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Human blood contains two cholinesterases. One is found in the plasma, and is unspecific so far as acetylcholine is concerned; it is therefore called pseudo-cholinesterase (Ps.ChE.). The other is the true cholinesterase of the red cells (Tr.ChE.). When succinylcholine was rediscovered by Bovet, Bovet-Nitti, Guarino, Longo, and Marotta (1949) and recommended as a muscle relaxant, its short action was already ascribed to its enzymatic destruction by the blood (Bovet-Nitti, 1949); this work was done on rabbits. Evans, Gray, Lehmann, and Silk (1952a) showed that, in man, of the two enzymes the pseudo-cholinesterase was responsible for the hydrolysis of succinylcholine. Bourne, Collier, and Somers (1952) found a significant difference in the pseudo-cholinesterase level when they compared six patients who had recovered normally from the effect of succinylcholine with six others whose response lasted beyond eight minutes. Evans *et al.* (1952a) demonstrated an inverse relationship between the duration of apnoea and the pseudo-cholinesterase level in the serum. It was suggested that unduly prolonged response to the drug should be treated with