TABLE I.
Serum Lipid in Rats Receiving Estradiol-benzoate (mg%)

Diet	Group	Total estradiol-benzoate (μg)	Total* lipid (mg%)	Avg
580-B	Controls ♂	—	363 403	351
	" ♀	—	386 251	
580-B	Sub-group 1 ♂	300 (3)†	366 301	334
	" " 2 ♂	720 (24)	578 553	566
	" " 3 ♂	120 (24)	521 541	531
560-B	♂	—	251	252
	♀	—	252	
550-B	♂	—	213	246
	♀	—	278	

*Each figure was obtained on the serum from 3 pooled samples of blood. Figures refer to total fatty acids plus total cholesterol.
†Figures in parentheses represent the number of injections.

Summary. A moderate lipemia was induced in male rats with subcutaneous injections of estradiol-benzoate. The animals were maintained on a diet rich in fat (71% hydrogenated coconut oil) but deficient in essential fatty acids.

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Physiological Properties of the Reynals Testicular Diffusion Factor.

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We have previously described[1] some chemical properties of the diffusion factor from mammalian testicle. This substance, discovered by Duran Reynals,[2] can be demonstrated by injecting a

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1 Aylward, PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 477.

2 Duran Reynals, *Compt. rend. Soc. Biol.*, 1928, **99**, 6.

small amount (*e. g.*, 0.2 ml) of an aqueous solution intradermally into the shaven skin of a rabbit; the extract spreads *immediately* over a large area, whereas a corresponding injection of saline or Ringer's solution remains localized as a bleb in the dermis for some time. The rate of disappearance of the bleb following the injection of an extract can be used as a rough test of activity. When an active extract mixed with an "indicator" (*e. g.*, India ink, hemoglobin, various dyes or diphtheria toxin) is injected intradermally, the indicator spreads over a large area; from such experiments methods of assay have been developed notably by Madinaveitia[3] and Bacharach[4] using hemoglobin. The present experiments were designed to investigate some physiological properties of the diffusion factor. It was also hoped to develop a more sensitive method of bioassay than was yet available.

Extracts were prepared as previously described[1] from the powder 4H, an acetone precipitate of an aqueous extract of beef testicles.

1. *Increase in Capillary Permeability after Intradermal Injection.* Five rabbits were given an intravenous injection of 5 ml of a 1% solution of the blue dye T. 1824. Each rabbit was then given 2 intradermal injections, one of 0.5 ml saline and a second of 0.5 ml of 5% saline solution of testicular extract (T.E.). Colored patches appeared in the skin round the site of the T.E. injections, showing that the dye was able to pass through the capillaries in the presence of the extracts, whereas at the control sites only a very slight coloration was seen. Similar results were obtained with intradermal injections in adult dogs and puppies.

The results, in agreement with those of Duran Reynals,[5] indicated that T.E. exercised a local effect on capillary permeability, but this cannot be regarded as a specific test for the diffusion factor as other substances producing local capillary damage have a similar effect.

2. *Increase in Capillary Permeability after Intravenous Injection.* Five ml of a 1% solution of T. 1824 were injected into the ear veins of each of 2 rabbits, followed by intravenous injections of 10 ml of 10% T.E. in one animal and 10 ml saline in the other. The experiments were repeated with further pairs of rabbits using 15 ml and 20 ml of T.E. The rabbits were killed 30 minutes after the injections.

The skins of all the rabbits were colored slightly at the end of 30 minutes, the coloration being particularly obvious in the ears and

[3] Madinaveitia, *Biochem. J.*, 1938, **32**, 1806.

[4] Bacharach, Chance and Middleton, *Biochem. J.*, 1940, **34**, 1464.

[5] Duran Reynals, *Yale J. Biol. and Med.*, 1939, **11**, 601.

nostrils, but the rabbits given T.E. developed skin coloration much more quickly and the final color was more intense. Similar observations were made on groups of mice given injections of 0.2 ml T.E. and 0.2 ml T. 1824 through the tail vein.

In agreement with the results of Duran Reynals[5] changes were also observed in the tissues of both rabbits and mice, the group given T.E. showing a pronounced degree of coloration in liver and lungs.

Experiments were then carried out in collaboration with Dr. M. I. Gregersen to see if this effect could be measured quantitatively, using a modification of the blood volume method of Gregersen.[6] In adult dogs (body weights about 2 kg) 2 ml of a 1% solution of the dye T. 1824 were injected into the jugular veins and blood samples were taken at intervals of 10 minutes over an hour. The dye content of the blood was measured, using a spectrophotometer and the results plotted graphically, the normal rate of loss of T. 1824 from the circulation being calculated for each animal. One hour after the dye injection, a sterile 5% solution of T.E. in amounts up to 200 ml was injected into the jugular vein over a 5-minute period. There were no obvious toxic symptoms. Blood samples were then taken at intervals over an hour and the dye concentrations plotted against time.

It was hoped that the effect of T.E. on permeability would be demonstrated by an increase in the gradient of curves, but apart from slight irregularities immediately following the injections there were no significant changes.

At the end of both experiments 0.25 ml injections of 5% T.E. were given intradermally to each dog, and within 5 minutes a blue ring appeared round the site of each injection, the central area being slightly red. The blue rings increased in size; the central area became smaller and in about 15 minutes had disappeared, so that a blue circular patch about 3.5 cm diameter resulted. The colored area continued to increase for some hours.

The experiments were repeated on puppies, no significant changes in the blood dye curve being found. 0.25 ml T.E. given intradermally to the puppies produced results similar to those in the adult dogs except that the skin colorations were even more marked.

The negative results following intravenous injection of the extracts may be due to partial inactivation of the beef testicular extracts in the blood stream of the dogs; they are more probably to be explained in terms of dosage. It was evident that no sensitive biological assay for T.E. could be developed on these lines.

[6] Gregersen, *J. Lab. and Clin. Med.*, 1938, **23**, 423.

3. *Experiments with the Clark Rabbit Ear Chambers.* In collaboration with Dr. R. G. Abell[7] experiments were performed on rabbits in the ears of which a moat chamber had been fitted, permitting microscopic observations of the effect on the capillaries of extracts placed in the moat. When testicular extracts (0.15 ml of 5% sterile solution of 4H) were placed in the moat, a striking increase took place in the permeability of the capillaries in the ear chamber, to such an extent that fluid passed through the vessel wall, leaving the corpuscles and part of the plasma concentrated within the capillaries. The concentration was sufficiently great to stop the flow of blood through some of the capillaries, but not sufficient to cause loss of outline of the corpuscles. The main effects were observed within 10 minutes and did not increase with continued contact of the extracts with the vessels.

In further experiments 142 mg/kg of T. 1824 was injected intravenously into each rabbit, and the rate of passage from the capillaries into the surrounding tissue was observed. Control studies by Abell[8] have shown that the dye cannot normally be seen outside the blood vessels until approximately 100 minutes after intravenous injection. When, however, 0.15 ml of sterile 5% T.E. were added to the moat its effect was readily seen under the microscope and within 13 minutes sufficient of the dye had passed out of the vessels to be readily visible in the tissues.

These experiments confirm in a very direct way the effects of testicular extracts on capillary permeability.

4. A number of other properties of testicular extracts have been studied. The ability of T.E. to increase absorption through the skin was tested by means of an insulin ointment in which T.E. was incorporated. Negative results were obtained. T.E. was found to be without effect on the contraction of the isolated guinea pig uterus and rabbit intestine. Injections of T.E. preparations produced no conspicuous changes in the blood pressure of experimental animals (dogs, cats, rabbits).

Since the above experiments were carried out, the observations of Chain and Duthrie[9] on the mucolytic activity of testicular extracts has led to the conception that the diffusion factor is identical or at least closely allied to hyaluronidase and that the diffusion is due to the action of this enzyme on the hyaluronic acid (a muco-polysaccharide)

[7] Abell and Aylward, *Anat. Rec.*, 1941, **79** (Suppl. 2), 1.

[8] Abell, R. G., *Anat. Rec.*, 1940, **78**, 214.

[9] Chain and Duthrie, *Brit. J. Exp. Path.*, 1940, **21**, 324.

present in normal skin and other tissues.[10] This recent work has been reviewed[10, 11] and will not be discussed here.

Conclusions. The experiments described indicate that testicular extracts have the property of increasing capillary permeability. No evidence was found that they could increase absorption from the skin, nor were pharmacological effects on smooth muscle or blood pressure demonstrable.

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Observations on Resistance of *Staphylococcus aureus* to Action of Tyrothricin.

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Tyrothricin, the crude substance isolated by Dubos from a spore-bearing soil bacillus has been found to kill gram-positive micro-organisms both *in vitro* and in the experimental animal.[1, 2] It is now known that tyrothricin contains 2 crystalline fractions, tyrocidine and gramicidin, the latter exhibiting the greater bactericidal action *in vitro* and *in vivo*.[3] Because of the difficulties encountered in preparing crystalline gramicidin, tyrothricin has been used almost exclusively in the treatment of infections.

Although tyrothricin is active against all gram-positive organisms, the different genera vary considerably in their susceptibility to its action. It is now well established that pneumococci and streptococci are most susceptible *in vitro* whereas staphylococci require somewhat greater concentrations of tyrothricin before a definite killing effect is observed.

During the past 2 years we have used tyrothricin in the treatment of experimental and clinical infections[2, 4] caused by the pneumococcus, streptococcus, and staphylococcus. Early in these studies the observation was made that it was difficult to predict from the *in*

10 Meyer and Palmer, *J. Biol. Chem.*, 1936, **14**, 689.

11 Madinaveitia and Quibell, *Biochem. J.*, 1940, **34**, 625.

12 McClean and Hale, *Biochem. J.*, 1941, **35**, 159.

1 Dubos, R. J., *J. Exp. Med.*, 1939, **70**, 11.

2 Rammelkamp, C. H., to be published.

3 Dubos, R. J., and Hotchkiss, R. D., *J. Exp. Med.*, 1941, **73**, 629.

4 Rammelkamp, C. H., and Keefer, C. S. (abstract), *J. Clin. Invest.*, 1941, **20**, 433.