Full reports of these investigations which are being continued will be published later.

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THE PREPARATION OF ADRENAL EXTRACT

SINCE we have received many requests for the details of our method of preparing an extract of the adrenal cortex, their publication seems desirable.

The medulla contains toxic substances[1] which must be removed if whole adrenals are to be used. We have tried to remove or destroy these substances by washing the ethereal solution with acid or alkali, or by treating the alcoholic or aqueous solutions with aluminum hydroxide, Lloyd's reagent, permutit, kaolin or charcoal. The toxic substances can be removed, but so much cortin is also removed that at present it seems more satisfactory to start with cortex alone.

Fresh adrenal cortex, or cortex from adrenals frozen immediately after killing, is finely ground in a meat chopper. Peroxide-free ethyl ether (a one per cent. $K_2Cr_2O_7$ solution acidulated with H_2SO_4 turns blue in a few seconds after shaking with peroxide ether) is added to the material in a flask as soon as possible. Ether must always be kept in the dark, because peroxides develop within a few hours upon exposure to light. We, therefore, work in a dark room or cover our flasks with opaque cloth. Peroxides destroy cortin and cause toxic substances to develop. A satisfactory proportion for extraction is 4 liters of ether to 3 kilograms of tissue in a 12 liter flask. The air in the flask is replaced with CO_2 and the flask closed with a rubber stopper wired in place. Extraction is facilitated by agitation on a shaker or rocker for four to eight hours. Care must be taken not to produce an emulsion by too vigorous shaking. After pouring off the ether extract, second and third extractions with ether are made in a like manner. The three ether extracts are combined and concentrated almost to dryness by vacuum distillation.

The residue from the ether distillation is extracted four times with 95–98 per cent. ethyl alcohol heated from 45° to 50° C. and kept warm during the extraction so that the fatty material is kept fluid, otherwise the alcohol does not penetrate. For one kilo of gland material about 50–60 cc of alcohol are used in each extraction. One hour on the shaker is adequate for each extraction. The flask is cooled by surrounding with cracked ice so that the alcohol may be easily separated from the oily matter. All fractions are combined and enough water added to make the alcohol content 80 per cent. The solution is chilled to −10° C. ($CaCl_2$ and ice mixture) or below and filtered in a cold atmosphere (4° C. or less) to remove undesirable material. This is extremely important. If the chilling is not sufficient or the solution becomes warmed during filtering, toxic substances are carried through the paper. The alcohol is removed *in vacuo* and the residue again extracted with a small volume of 60–75 per cent. alcohol, then chilled and filtered as above. The alcohol is again removed and the residue extracted with a small volume of ether. The ether is driven off and its residue taken up with sufficient water to make the desired concentration. NaCl is added to make the extract isotonic with the body fluids. After passing through a Seitz filter, the extract is ready for injection.

If the precautions are carefully observed a potent, non-toxic extract can be made. One patient was injected four times daily for more than seven months without untoward effects.

Recently, Britton and Silvette[2] compared our method with that of Swingle and Pfiffner. One of the investigators from Britton's laboratory spent several weeks working with Swingle and Pfiffner,[3] while their knowledge of our method was based only upon the brief published[4] outline of our process. The results of Britton and Silvette are, therefore, what one might expect. Perla and Marmorston-Gottesman[5] have recently used our method successfully. Dr. Perla spent two days in our laboratory.

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THE LIFE HISTORY OF BABESIA BIGEMINA IN THE NORTH AMERICAN FEVER TICK

Babesia bigemina (Smith and Kilbourne, 1893)[1] is the piroplasm which is the causative agent of Texas cattle fever, a disease that at one time threatened the existence of a cattle industry in the United States. Smith and Kilbourne (1893) demonstrated conclu-

[1] E. B. McKinley and N. F. Fisher, *Am. J. Physiol.*, 76: 268, 1926.

[2] S. W. Britton and H. Silvette, SCIENCE, 73: 322, 1931.

[3] W. W. Swingle and J. J. Pfiffner, *Am. J. Physiol.*, 96: 153, 1931.

[4] F. A. Hartman, K. A. Brownell and W. E. Hartman, *Am. J. Physiol.*, 95: 670, 1930.

[5] D. Perla and J. Marmorston-Gottesman, *Proc. Soc. Exp. Biol. and Med.*, 28: 650, 1931.

[1] T. Smith and F. Kilbourne, *Bull.* Bur. Animal Industries, U. S. Dept. Agr., Washington, 1: 177–304, 1893.

sively that *Babesia bigemina* is transmitted from host to host only by the agency of *offspring* of female ticks which had previously fed upon the blood of cattle which at the time of feeding were suffering, or had recovered, from an attack of Texas fever. *Margaropus annulatus* (Say) is the obligatory vector of this protozoan parasite in North America, and the life history of *Margaropus,* a one-host tick, makes the life history of *B. bigemina* inseparable from the developmental cycle of the tick.

Ticks for this investigation were collected by the writer from dairy cattle east of Texarkana, Arkansas, during the summer of 1928, from near Abbeville, Louisiana, in July, 1929, and a bountiful supply has been provided through the courtesy of Dr. C. W. Rees, of the zoology division of the Bureau of Animal Industry, Iberia Experiment Farm, Jeanerette, Louisiana. The observations reported here were made on two series of ticks: (1) the gut contents, organs, ova, embryos and newly hatched larvae of female ticks which had fed on the blood of a bull suffering from an acute attack of Texas fever; (2) a similar series of control ticks reared on a mule to clean them, and on clean susceptible steers for three subsequent generations. The bites of larvae belonging to series No. 1 produced the disease in a susceptible animal, the host reacting on the fourteenth day after being infested.

The parasites were studied in fresh saline preparations, smears and sections.

The most complete set of observations on the life cycle of a piroplasm heretofore published is the work of Christophers (1907)[2] on the life cycle of *B. canis.* A preliminary report on the life cycle of *Theileria parva* was recently published by Cowdry and Ham (1930).[3] Several of the forms described by Christophers as stages of *B. canis* appear also in the development of *B. bigemina.*

The sexual phenomena and sporogony of *Babesia bigemina* take place in the tick. The early phases occur in the lumen of the gut after the ingestion of parasitized blood.

1. Examination of gut contents reveals: (*a*) typical trophozoites (Dennis, 1930)[4] that have recently been taken into the gut and which may be free in the ingested mass, or may be incorporated in the wandering digestive macrophage cells of the tick; several may accumulate in a single macrophage. Parasites that are so phagocytized are soon digested. Pear-shaped and rounded forms of the parasites are observed free in the gut. (*b*) A leaf-shaped form of the parasite that is quite abundant. This form stains poorly and is believed to be degenerate. (*c*) There is present a very characteristic club-shaped form. These bodies are *isogametes.*

2. The gametes are club-shaped parasites about 5.5 to 6.0 μ in length. They arise by growth and modification of structures from certain trophozoites that are indistinguishable from other trophozoites. The male gametes are not distinguishable from the female gametes. These sexual forms are actively motile.

3. Fertilization apparently takes place by the isogametes becoming associated first at the blunt anterior end, and then fusing. Syngamy results in the formation of a motile zygote or oökinete which soon leaves the lumen of the gut.

4. The öokinetes penetrate the tissues of the thin gut wall where they may round up, grow and form *sporonts* ranging from 5.0 to 12.0 μ in diameter, or they may pass completely through the gut wall and enter the ovary where they invade the ova.

5. In the ovum the oökinete also forms a sporont, which then divides to form multinucleate "sporoblasts." These sporoblasts are amoeboid and actively motile, and migrate throughout the embryonic tissues of the developing tick. Because of their migratory nature these somatellas are called *sporokinetes.* The sporokinetes are very pleomorphic and have from four to about thirty-two nuclei. During the ontogeny of the tick the sporokinetes may come to infest almost any tissue of the embryo.

6. Since much of the anterior embryonic cell mass of the tick is destined to contribute to the salivary glands, some of the alveoli come to be occupied by sporokinetes. As the incubation period of the tick draws to a close, some of the sporokinetes undergo multiple fission or "sporogony" to form *sporozoites;* others may not form sporozoites until after the tick has hatched.

7. The sporozoites are miniature trophozoites, and may also multiply, doing so by the "budding" process which is characteristic of the trophozoite. The sporozoites are inoculated into the blood stream of the bovine host in the saliva of the feeding seed-tick, and soon set up an active infection of the erythrocytes of the host.

The writer wishes to express here his sincere thanks to the kindness and generosity of Dr. Rees, who provided material of known infectivity and lineage, and to Professor C. A. Kofoid, of the Department of Zoology, University of California, at Berkeley, under whose supervision this research has been carried on.

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[2] S. R. Christophers, *Scient. Mem. Govt. of India,* n. s., 29: 1–83, 1907.

[3] E. V. Cowdry and A. W. Ham, SCIENCE, 72: 461–462, 1930.

[4] E. W. Dennis, Univ. Calif. Publ. Zool., 33: 179–192, 1930.