TABLE 2

EFFECT OF STROPHANTHIN ON SOME BLOOD CONSTITUENTS OF CATS

| Cat | Sugar mg per cent. | | | Potassium mg per cent. | | | Protein gm per cent. | | |
|---|---|---|---|---|---|---|---|---|---|
| | Initial | Max. change | Time* | Initial | Min. value | Time* | Initial | Min. value | Time* |
| 30 gamma per kilo | | | | | | | | | |
| A ... | 111 | +17 | 45 | 22.0 | 20.0 | 15 | 7.58 | 6.02 | 75 |
| B ... | 100 | +45 | 15 | 21.0 | 19.9 | 15 | 7.21 | 6.02 | 90 |
| C ... | 71 | +25 | 10 | 27.6 | 20.8 | 10 | 7.41 | 7.08 | 30 |
| D ... | 50 | +37 | 45 | 20.5 | 18.6 | 15 | 8.50 | 6.85 | 90 |
| 50 gamma per kilo | | | | | | | | | |
| E ... | 61 | +35 | 45 | 25.1 | 19.9 | 5 | 7.06 | 6.50 | 105 |
| F ... | 58 | +34 | 60 | 27.3 | 21.3 | 5 | 7.06 | 6.64 | 75 |

* Times given are in minutes from injection to greatest change.

ous administration of cardiac glycosides, and that the blood sugar level of normal cats can be significantly elevated by injection of these glycosides.

Both effects are similar to those obtained with adrenal cortex extract.

In view of the cortin-like effect of steroid glycosides on potassium, previously reported by us,[9] it is important to know that the same crystalline substances will also affect carbohydrate distribution and counteract the convulsive action of insulin. The fact that in some experiments the protection was effective when given 18 hours or more before insulin is particularly noteworthy.

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## A PHYSIOLOGICALLY ACTIVE PRINCIPLE FROM CANNABIS SATIVA (MARIHUANA)

WHILE it has long been known that the physiological activity of *Cannabis sativa* (marihuana or hashish) is associated with its contained resins, no physiologically active crystalline material has heretofore been isolated. We report in this note the isolation of such a substance.

The hydrocarbon nonacosane and an oily product termed *cannabinol* were first isolated by Wood, Spivey and Easterfield.[1] In 1938 Bergel, Todd and Work[2] reported the preparation of a crystalline p-nitro benzoate of cannabinol which could be used to separate the cannabinol from the oil by chromatographic adsorption methods. Recently an oily product which was named *cannabidiol* was isolated by Adams, Hunt and Clark.[3] None of these well-defined products has exhibited the characteristic physiological activities that are shown by the crude drug though *cannabinol* was found to be quite toxic. Reviews of the earlier work on the chemistry of *Cannabis* have been published by Walton[4] and by Blatt.[5]

Work on the separation of physiologically active fractions from alcoholic extracts of *Cannabis sativa* has been in progress for the past year in our laboratories. The extracts of Minnesota wild hemp used for the work were generously supplied by the Narcotics Laboratory, United States Treasury Department, and we are indebted to Messrs. H. J. Anslinger and H. J. Wollner for their collaboration which made this work possible.

The alcohol extract of the crude drug was diluted with water to yield a seventy per cent. alcohol solution, and this was partitioned into petroleum ether. Salt-forming compounds were extracted and then colored substances were largely removed by adsorption on zinc carbonate. The resultant resinous material was fractionally precipitated from methanol with water and there was obtained a physiologically active fraction of about one twentieth the weight of the crude resin material. This purified product was fractionally distilled under 0.005 mm pressure, with the most active fraction distilling at 128°–135° C. This fraction is a red-colored oil which shows typical activity in dogs following an oral dose of 1.0 mg per kg. By cooling a solution of this oil in a methanol-acetic acid mixture, some crystalline material was obtained. This was then recrystallized several times from methanol to yield colorless needles melting at 128°–129° C.

When crystalline material thus isolated was administered orally to a dog in a dose of 0.1 mg per kg, incoordination of movements followed after two hours and persisted for about four hours. A dose of 0.38 mg per kg was administered to the same dog three days later. Within two hours incoordination of movements was apparent and this effect persisted notably for six hours. A transitory catatonic depression was also observed, during which the animal gazed fixedly at particular objects for long periods of time; breathing was deep and dyspneic, and cardiac arrhythmia was noted. Fibrillary tremors of the left leg were observed as were, also, periods in which the dog engaged in vigorous though seemingly useless scratching. The dog used for these studies had been standardized for response to the crude drug material from which the crystalline material was prepared and had shown similar effects to those above noted following a dosage of 20 mg per

[9] R. L. Zwemer and B. E. Lowenstein, SCIENCE, 91: 75, 1940.

[1] Wood, Spivey and Easterfield, *Jour. Chem. Soc.*, 69: 539, 1896; 75: 20–36, 1899.

[2] Bergel, Todd and Work, *Chem. and Ind.*, 57: 86, 1938.

[3] Adams, Hunt and Clark, *Jour. Am. Chem. Soc.*, 62: 196, 1940.

[4] R. B. Walton, "Marihuana." Pp. 223. J. B. Lippincott and Company., 1938.

[5] A. H. Blatt, *Jour. Wash. Acad. Sci.*, 28: 465, 1938.

kg but a dosage of 10 mg per kg was ineffective. The crystalline material thus appears to be more than one hundred times as active as crude drug material.

For convenience we have named the compound *cannin* and the suffix *-in* may be changed later so as to conform to standard chemical nomenclature when more is known of the chemical structure.

The alkaline Beam test, used in the forensic detection of *Cannabis sativa* resin materials, was tried on *cannin* and found to be completely negative. The distillation fractions and the mother liquors from which the *cannin* was obtained gave positive tests.

Work is continuing on *Cannabis* to isolate larger quantities of this active principle for structure determination and more extensive physiological studies. A search is being made for other active principles which may be present.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## ON THE USE OF CHICK EMBRYO CULTURES OF INFLUENZA VIRUS IN COMPLEMENT FIXATION TESTS[1]

MOST of the complement fixation studies in influenza have been carried out with antigens prepared from infected mouse lung tissue, either fresh or desiccated.[2-11] Minced chick embryo cultures (Smith,[11] Tulloch[8]) and infected chorio-allantoic membrane inoculated by the Goodpasture[12] method have also been used (Hoyle and Fairbrother,[13] Lush and Burnet[14]) but with variable results.

When Cox[15] reported that the direct inoculation of rickettsiae of the Rocky Mountain spotted fever and typhus groups into the yolk of the developing chick embryo resulted in the multiplication of these organisms in the yolk sac to a high concentration, it seemed that this method might lend itself to the cultivation of the influenza virus as a more satisfactory source of complement fixation antigen as well as virus. Using the method of Cox, the PR8 strain of influenza virus killed the embryos within one or two days after which autolysis proceeded rapidly. It seemed possible that the virus might also multiply in the yolk sac with a delay in lethal effect, if it were introduced at a point outside the yolk. The virus was, therefore, inoculated between the yolk sac and the chorio-allantoic membrane, passing the needle through a small hole in the shell at the air sac end of the egg. By this method a high concentration of virus occurred in the membrane surrounding the yolk. There was sufficient virus in 0.1 cc of $10^{-4}$ and $10^{-5}$ dilutions of yolk sac tissues to produce fatal infection in both embryos and mice. When the chorio-allantoic and amniotic membranes of eggs, inoculated in the same way, were pooled and titrated, the virus titer of these combined tissues was found to be about 10× higher than that of the yolk sac and 100× higher than that of chorio-allantoic membrane inoculated by the Goodpasture method. The details of this particular phase of the study on the cultivation of influenza virus will appear in another publication.

The yolk sac and the pooled chorio-allantoic and amniotic membranes of embryos inoculated as described above were found, moreover, by comparative titration of these tissues and mouse lung suspensions to be good sources of complement fixing antigen for serological purposes. Antigens prepared from these tissues were comparable to antigen in mouse lung in both complement-fixing activity and specificity, as will be seen from the results in Table I. Eggs inoculated with the PR8 strain as described were incubated at 37° for 2 or 3 days. The membranes were then separated, washed in saline and drained on filter paper. The tissues were ground to a paste with alundum and saline added to make 10 per cent. suspensions. For comparison 10 per cent. suspensions were similarly prepared from the lungs of mice inoculated three days previously with the same strain of virus. The antigens were clarified as much as possible in the centrifuge and serial dilutions were titrated against serial dilu-

[1] These investigations were financed largely by a grant from the International Health Division of the Rockefeller Foundation.

[2] R. W. Fairbrother and L. Hoyle, *Jour. Path. and Bact.*, 44: 213, 1937.

[3] L. Hoyle and R. W. Fairbrother, *Brit. Med. Jour.*, 1: 655, 1937.

[4] *Ibid.*, *Jour. Hyg.*, 37: 512, 1937.

[5] Thomas Francis, Jr., T. P. Magill, E. R. Rickard and M. Dorothy Beck, *Am. Jour. Pub. Health*, 27: 1141, 1937.

[6] R. W. Fairbrother and A. E. Martin, *Lancet*, 1: 718, 1938.

[7] Allison P. Morrison, Dorothy R. Shaw, Athol S. Kenney and Joseph Stokes, Jr., *Am. Jour. Med. Sci.*, 197: 253, 1939.

[8] W. J. Tulloch, *Edinburgh Med. Jour.*, 46: 117, 200, 278, 340 and 415, 1939.

[9] A. E. Martin, *Jour. Hygiene*, 40: 104, 1940.

[10] M. D. Eaton and E. R. Rickard, in press.

[11] Wilson Smith, *Lancet*, 2: 1256, 1936.

[12] Ernest W. Goodpasture, *Am. Jour. Hygiene*, 28: 111, 1938.

[13] L. Hoyle and R. W. Fairbrother, *Brit. Jour. Exp. Path.*, 18: 425, 1937.

[14] Dora Lush and F. M. Burnet, *Australian Jour. Exp. Biol. and Med. Sci.*, 15: 375, 1937.

[15] Herald R. Cox, *Pub. Health Rep.*, 53: 2241, 1938.