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.

A. J. HAAGEN-SMIT
C. Z. WAWRA
J. B. KOEPFLI
G. A. ALLES
G. A. FEIGEN
A. N. PRATER
CALIFORNIA INSTITUTE OF TECHNOLOGY

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.

TABLE I

COMPARISON OF EGG MEMBRANES AND MOUSE LUNG AS ANTIGENS IN COMPLEMENT FIXATION TESTS

Antigen				Convalescent serum diluted					Normal serum diluted			
Normal tissue	Infected tissue	10 per cent. susp. diluted		1–4	1–8	1–16	1–32	1–64		1–4	1–8	1–16
	Yolk sac No. 1	1–16	S.R.	++++	++++	++++	++++	+++	S.R.	0	0	0
	C.A.–A. No. 1	1–16	"	++++	++++	++++	++++	++	"	0	0	0
	C.A.–A. No. 2	1–8	"	++++	++++	++++	++++	++	"	0	0	0
	Mouse lung No. 1	1–8	"	++++	++++	++++	++++	+++	"	0	0	0
Yolk sac		1–8	"	0					"	0		
C.A.–A.		1–8	"	0					"	0		
Mouse lung		1–8	"	0					"	0		
	Yolk sac No. 1	1–16	J.E.	++++	++++	++++	+++	+	J.E.	0	0	0
	" " No. 2	1–8	"	++++	++++	++++	+++	+	"	0	0	0
	C.A.–A. No. 1	1–16	"	++++	++++	++++	+++	0	"	0	0	0
	" No. 2	1–8	"	++++	++++	++++	+++	0	"	0	0	0
	Mouse lung No. 2	1–8	"	++++	++++	++++	+++	+	"	0	0	0
Yolk sac		1–8	"	0					"	0		
C.A.–A.		1–8	"	0					"	0		
Mouse lung		1–8	"	0					"	0		
	Yolk sac No. 1	1–16	Pos.* Con.	++++	++++	++++	++++	+++	Neg. Con.	0	0	0
	" " No. 2	1–8	"	++++	++++	++++	++++	++	"	0	0	0
	C.A.–A. No. 1	1–16	"	++++	++++	++++	++++	+++	"	0	0	0
	" No. 2	1–8	"	++++	++++	++++	++++	+++	"	0	0	0
	Mouse lung No. 1	1–8	"	++++	++++	++++	++++	+++	"	0	0	0
	" " No. 2	1–8	"	++++	++++	++++	++++	+++	"	–	–	–
Yolk sac		1–8	"	0					"	0		
C.A.–A.		1–8	"	0					"	0		
Mouse lung		1–8	"	0					"	0		

* Pos. Con. consisted of 6 pooled convalescent sera.
Neg. Con.—Normal serum.
C.A.–A.—Pooled chorio-allantoic and amniotic membranes.
Test: 0.2 cc serum dilutions.
0.2 [illegible] diluted 1-20 (2 units).
0.2 [illegible]
After [illegible] 7° water bath, 0.5 cc sensitized cells was added and the tests incubated for 30 minutes.
++++—Complete fixation. 0—No fixation.
None of the sera was anti-complementary in 1–4 dilution.
None of the antigens was anti-complementary in 1–8 dilution.

tions of human convalescent serum to determine the optimal antigenic activity of each suspension. Complement fixation tests were carried out with the optimal antigenic dose of each suspension and human sera, convalescent and normal. The egg membrane and mouse lung antigens were entirely comparable in activity. Two suspensions similarly prepared from the embryos themselves were devoid of complement-fixing activity.

The chorio-allantoic and amniotic membranes have the following special advantages as complement-fixing antigen: (*a*) A relatively clear solution is obtained by centrifuging the saline suspensions of the membranes. (*b*) The membranes from one egg yield approximately 100 cc of antigen, diluted ready for use, or as much antigen of the same or better activity as the lungs from 6 or 7 mice yield. (*c*) These egg antigens can be used with ferret serum, whereas mouse lung can not because of the heterophile reactions between ferret serum and normal mouse lung.[2]

A further elaboration of the studies in this preliminary report will appear in a subsequent publication.

CLARA NIGG
JAMES H. CROWLEY
DORIS E. WILSON

INFLUENZA RESEARCH LABORATORY,
MINNESOTA STATE BOARD OF HEALTH,
MINNEAPOLIS

BOOKS RECEIVED

BOGGS, S. WHITTEMORE. *International Boundaries; a Study of Boundary Functions and Problems.* Pp. xvii + 272. 7 plates. 26 figures. Columbia University Press. $3.25.

Bulletin Analytique; Centre National de la Recherche Scientifique. Vol. I, Nos. 1–6, Janvier-Mars, 1940. Vol. I, Nos. 7–8, Avril, 1940. Service de Documentation du C.N.R.S., Paris.

CASIMIR, H. B. G. *Magnetism and Very Low Temperatures.* Pp. 93. 14 figures. Cambridge University Press, Macmillan. $1.40.

GAMOW, GEORGE. *The Birth and Death of the Sun.* Pp. xiv + 238. 60 figures. 16 plates. Viking Press. $3.00.

JACOBS, MELVILLE. *Coos Myth Texts.* Pp. 259. University of Washington, Seattle.

PAULING, LINUS. *The Nature of the Chemical Bond.* (The George Fisher Baker Non-resident Lectureship in Chemistry at Cornell University.) Pp. xvi + 450. Illustrated. Cornell University Press. $4.50.

PORTERFIELD, JOHN and KAY REYNOLDS, Editors. *We Present Television.* Pp. 298. Illustrated. Norton. $3.00.

Rothamsted Experimental Station. *Library Catalogue of Printed Books and Pamphlets on Agriculture Published between 1471 and 1840.* Second edition. Pp. 293. The Station, Harpenden, England. 15/8.

THORNE, P. C. L. and A. M. WARD, Editors. *Fritz Ephraim's Inorganic Chemistry.* Third English edition. Pp. xii + 911. 98 figures. Nordeman. $8.00.

TIMOSHENKO, S. and GLEASON H. MACCULLOUGH. *Elements of Strength of Materials.* Second edition. Pp. xii + 365. 365 figures. Van Nostrand. $3.25.

VON MISES, RICHARD. *Library of Unified Science: Kleines Lehrbuch des Positivismus; Einführung in die Empiristische Wissenschaftsauffassung.* Pp. xii + 467. University of Chicago Press. $5.50.