The A factor produces a definite reduction in the lag period and a stimulation of the initial growth. We have obtained no growth in its absence, and it appears furthermore to be needed by cells in a resting condition. It has a small molecular size. Electrodialysis shows it to be an acid. It can be precipitated with calcium or copper with subsequent recovery of activity. It is only slowly destroyed at 100° C., providing the pH is neutral.

Thus the dormancy of adult tissues appears to involve a balance between the non-diffusible "tissue inhibitor," on the one hand, and the stimulating A factor and proteolytic enzymes on the other hand (and probably other agents).

The physiological condition of adult tissue cells seems similarly to involve a balance between certain hormone-like "controlling agents." There are at least four of these in plasma, the A, B, C and D factors.

(8) The first of these, the A factor, is not only a stimulant, as mentioned above, but it appears furthermore to be needed by dormant cells. If a culture of adult fibroblasts is washed repeatedly with serum ultrafiltrate (containing the A factor) the B, C and D factors are thereby removed without depriving the cells of the needed A factor. The cells then become clear, stellate and free from fat granules. They can be kept in a healthy state indefinitely, merely by semi-weekly washing with serum ultrafiltrate.

(9) When one of these cultures of clear cells is treated with a solution containing the B factor (obtained from chicken plasma or dog serum) the cells become filled with fat granules. These can be seen in 24 hours and are very conspicuous in two or three days. This process can be reversed. Repeated washing with serum ultrafiltrate results in a complete absorption of the fat granules. Thus the B factor is an agent which causes the cells to produce fat granules—but it does not produce degeneration.

(10) The C factor, however, produces degeneration but does not produce fat granules. It is closely associated with the B factor. This degeneration is not reversible.

(11) There is also evidence for a "D factor" which produces cohesiveness between fibroblasts. The cultures which have been washed with serum ultrafiltrate contain independent isolated cells. On the addition of certain fractions these cells coalesce to form the usual reticulum.

(12) Segments of chicken innominate arteries were incubated in solutions containing the B factor. Frozen sections stained with Scharlach R showed that fat had been deposited in these arteries *in vitro*. The fat was seen as a thin layer along the intima with scattered droplets in the adjacent media. This same distribution of fat occurs spontaneously in chicken arteries *in vivo*.

Details will be published elsewhere. Further work is in progress.

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## BACTERIOLOGIC EXPERIMENTATION ON THE GUINEA PIG FETUS

ALTHOUGH bacteriologists are continually in search of new and more adaptable experimental animals, the possibility of using the fetus for bacteriologic studies appears to have been almost entirely overlooked. Most of the recorded investigations dealing with experimental fetal infections have been based on the passage of the infectious agent from maternal to fetal circulations through the placenta. Obviously, in such experiments there can be no control of the time of effective fetal inoculation or of the amount of inoculum which actually reaches the fetus. That direct manipulation of the mammalian fetus is possible, however, has been shown by Bors,[1] Wohlwill and Bock,[2] and others. Their studies have been concerned chiefly with developmental processes and pathologic changes. The latter authors studied particularly the cellular type of response of the guinea pig fetus to directly inoculated chemical and bacterial irritants.

The potential value of the fetus as an experimental animal for bacteriologic purposes is based on its inherent sterility and on the possibility that the fetus may present useful variations in susceptibility to certain disease agents in comparison with the postnatal representative of the same species. This possibility may be inferred from the fact that the fetus differs markedly from the postnatal animal not only in size and structure, but in physiological and biochemical processes as well. In order to investigate this possibility and to learn some of the technical applications and limitations in the use of the fetus as an immediate experimental animal for bacteriologic research, we[3] have inoculated fetal guinea pigs *in utero* with six infectious or toxic agents. They were selected to represent a wide range of host-parasite relationships, *viz.*, the poliomyelitis virus, for which the monkey is at present the only susceptible experimental animal; the vaccinia virus, which finds a relatively resistant host in the guinea pig; diphtheria toxin, for the study of which the guinea pig may be said to be the classic experimental animal; two strains of the tubercle bacillus (H37 and BCG), representing, respectively, bacteria virulent and relatively non-virulent for guinea pigs and other animals; and the submaxillary gland

[1] E. Bors, *Archiv. f. Entwick. d. Org.*, 105: 655, 1925. *Ibid., Deutsche Ztschr. f. Chir.*, 203–204: 669, 1927.

[2] F. Wohlwill and H. E. Bock, *Virchow Archiv. f. path. Anat. u. Physiol.*, 291: 864, 1933.

[3] *Am. Jour. Pathol.*, in press.

virus of guinea pigs, a virus natural to that animal and latent in spontaneous infections.

The most favorable fetal age for such inoculations was found to be 30–35 days. Earlier than this the fetuses are too small to be inoculated directly; older fetuses are more likely to be aborted. Since the gestation period of the guinea pig is from 60 to 65 days, inoculation of the fetuses about the thirtieth day provides about a month of possible intrauterine development, following inoculation. Fetal inoculations were made principally intracerebrally by needle puncture through the maternal uterine wall and fetal membranes, after surgical exposure under ether anesthesia. Experiments were terminated usually by delivery of the fetuses through cesarean section after an appropriate incubation period, although some of the mothers were allowed to go to term in cases where a longer experimental period was preferred.

The poliomyelitis virus did not affect the vitality of the fetuses nor was there any determinable histologic reaction following intracerebral inoculation. Two attempts at serial passage, one through eight transfers at 5-day intervals, the other through four transfers at 10-day intervals, were also unproductive. The passage material proved non-infectious for monkeys at the end of each series. Thus the known resistance of the species to this virus obtained likewise in the fetus under the conditions provided.

The guinea pig fetus proved to be particularly susceptible to the vaccinia virus in contrast to the characteristically sluggish reaction of mature guinea pigs. Quantities of virus that caused scarcely a visible reaction in postnatal animals were sufficient to evoke wide-spread cutaneous and visceral lesions and even death in the fetus. Identification of the virus in fetal tissues was made by intradermal tests in immune and non-immune rabbits. Although the virus was administered to the fetuses intracerebrally, the fetal brain usually contained little virus at the termination of the experiment. The kidneys consistently gave high titers, and lung and skin lesions likewise were dependable sources of virus. The virus was recovered from only two of ten specimens of heart blood. Nine serial passages through fetuses did not alter the properties of the virus in respect to the rabbit and the guinea pig.

Although the guinea pig is a classic test animal for diphtheria toxin, many workers have held that very young animals are relatively more resistant. In our experiments, fetal guinea pigs reacted acutely to the toxin, and the minimal lethal doses for fetal and postnatal animals were about the same when calculated on the basis of toxin per gram body weight.

We have found the fetal guinea pig delicately responsive to small dosages of the tubercle bacillus given intracerebrally. Both the virulent strain (H37) and the allegedly non-virulent strain (BCG) caused extensive and progressive pathologic changes comparable to those seen in adult animals following the inoculation of virulent organisms. H37, however, was effective in smaller amounts and disseminated with more facility. Metastatic lesions, demonstrable grossly and microscopically and containing acid-fast organisms, were commonly found in the spleen, liver and lung following inoculation of small quantities (0.01 mgm) of H37 and larger dosages (0.1–1.0 mgm) of BCG. Death regularly resulted in all the fetuses injected with H37 and in those receiving the larger quantities of BCG. Recovery of BCG on media was irregular, and fetus-to-fetus transfer was not accomplished in the few attempts made.

The submaxillary gland virus disseminated from the cerebral site of inoculation much more widely in the fetus than in new-born animals, and cellular inclusions were numerous in various organs, particularly in the meninges, liver and placenta. It is of theoretical interest that the fetuses of mothers immune to the virus were just as susceptible as those carried by non-immune mothers and were readily killed *in utero* by the action of the virus.

We believe that both the practicability and potential value of bacteriologic experimentation on the mammalian fetus are established by these experiments. The principal difficulties encountered are those associated with the problem of inoculating the fetus without disturbing gestation, and the problem of following the progress of the infection after inoculation. By using fetuses at the optimal age, one can largely avoid abortions; experience with the particular virus and animal, in preliminary tests, will be the guide in judging dosages and incubation periods. The obvious technical advantage in the use of the fetus is that it is inherently sterile and occupies an environment of constant temperature and nutrition. When there are several fetuses in the litter an excellent opportunity is provided for controls and for a graded series of the inoculum. In our experience there has been no transfer of inoculum from one fetus to another within the same litter. A point of interest in such work is that the fetus *in utero* may tolerate more severe pathologic changes than are compatible with life in the outer world. So long as the placenta is intact the fetus may survive in the entire absence of brain, lung and other organs essential for independent existence. Thus the fetus may provide for observation more extensive lesions than could occur in the living postnatal animal. In our studies, pathologic changes characteristic for the agent were induced in each case, except with the poliomyelitis virus, and the range of reaction, dependent on dosage, varied from a

faintly perceptible lesion to extensive tissue destruction and death.

Cultivation in tissue culture and in the chorio-allantoic membranes of the chick has demonstrated that under such conditions embryonic tissue is peculiarly susceptible to a variety of infectious agents, particularly filterable viruses. That a similar situation exists with respect to the mammalian fetus *in utero* may be inferred from our studies. Certainly the susceptibility of the guinea pig fetus may exceed that of the postnatal representative of the same species, as we have seen especially in connection with the vaccinia virus. It is reasonable to expect also that fetal reactions to infectious agents will be found in certain cases to differ not only in degree but also in kind. In these respects, then, the fetus may be said in effect to constitute a new experimental animal for bacteriologic procedures.

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## THE ISOLATION FROM COTTONSEED OIL OF AN ALCOHOL RESEMBLING ALPHA TOCOPHEROL FROM WHEAT-GERM OIL

EVANS, Emerson and Emerson have reported the isolation from wheat-germ oil of an alcohol, alpha tocopherol, having the properties of vitamin E.[1]

The same procedure has been followed in the preparation of the corresponding alcohol from cottonseed oil. Olcott[2] has demonstrated that a biologically potent concentrate could be prepared from cottonseed oil. Although this oil has only 0.7 per cent. non-saponifiable matter, as compared with 5.0 per cent. for wheat-germ oil, the commercial production of cottonseed oil makes it a readily available source material.

Four allophanates were isolated:

(1) m.p. 240°—regenerated alcohol biologically inactive as vitamin E.
(2) m.p. 158°–160°—regenerated alcohol, biologically active, believed to be identical with alpha tocopherol.
(3) m.p. 134°–135°—regenerated alcohol biologically active. Further investigation to be made.
(4) m.p. 80°—regenerated alcohol biologically inactive.

The 158°–160° allophanates from cottonseed oil and wheat-germ oil appear to be identical for the following reasons: (1) The two compounds have the same melting point; (2) There is no depression in mixed melting points; (3) Both compounds exhibit a maximum absorption in the ultra-violet between 2,900 and 3,000 Ångstrom units; (4) The alcohols regenerated from the two allophanates show similar biological activity.

An attempt is being made to isolate one or more of these compounds from a lettuce-oil concentrate.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## A FURTHER IMPROVED PROSPECTING PICK

BROWN described[1] an improved prospecting pick as ". . . a perfect tool of the kind, of drop forged highest grade 85 carbon tool steel, with a perfect eye extended so as to secure the full purchase power of the handle. . . ." The writer thoroughly tested one of these picks on various formations in the Blue Ridge and Massanutten mountains in Virginia in the summer of 1932. We agree with Brown that the pick has good dimensions and balance, and that it is a serviceable tool. It may be all that is desired for certain kinds of work, but "perfect" is a descriptive term which should be rarely used.

We believe we have improved upon this perfect tool by applying a relatively small amount of a very hard alloy of cobalt-chromium-tungsten,[2] which serves as the cutting or digging edge for both the spatula and pointed end of the pick. On the point of the pick the alloy was applied only to the outer triangular face, the one opposite the handle. On the spatula end only the outer face was treated, and here the hard-facing material was carried to the chisel point. The location of the hard-facing material on the ends of the pick in this fashion resulted in a saving of material—approximate thickness 1/16 inch—and also made the

[1] H. M. Evans, O. H. Emerson and G. A. Emerson, *Jour. Biol. Chem.*, 113: 319, 1936.

[1] B. Brown, SCIENCE, 75: 291, 1932.

[2] H. S. Olcott, *Jour. Biol. Chem.*, 107: 471, 1934.

[2] Haynes Stellite is the trade name applied to an alloy of cobalt-chromium-tungsten. One pick was very kindly modified, as described, by the Haynes Stellite Company, Kokomo, Indiana.