Whereas the presence of circulating pituitary gonadotrophin is the *sine qua non* of ovarian stimulation, in the presence of adequate amounts ovarian activity seems to largely be regulated by the level of circulating estrogens present.

Summary. Both ovaries of mature female rats were autotransplanted into the spleen, allowing the ovaries to be stimulated by pituitary gonadotrophins, but denying them the presence of circulating estrogen by interposing the liver between the ovaries and the systemic circulation.

The ovaries of these animals increased 3-fold in weight after 30-57 days transplantation (149.5 mg) over their weight at the time of operation (49.0 mg).

Two groups of experimental animals were injected with estradiol benzoate. One group received amounts exceeding physiological requirements and the other amounts approximately meeting physiological requirements. In both groups, the ovarian weights showed a marked decrease below the weight at operation.

It is concluded that ovarian growth is inhibited by the presence of circulating estrogens.

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Bodies Suggesting Exoerythrocytic Forms of *Plasmodium vivax* in Tissue Culture.*

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The demonstration of exoerythrocytic forms of avian malarial parasites[1] has stimulated a search for similar bodies in mammalian malaria. A few authors have described structures which they believed to be exoerythrocytic forms of human malarial parasites. The subject has been reviewed by Porter and Huff[2] and by Brug.[3] Brug[3] himself presented figures of bodies seen in smears of the lungs of a patient dying in the acute stage of *Plasmodium vivax* infection which he thought to be exoerythrocytic forms. Recently Raffaele[4] has reiterated the claim that he has seen such forms in all 3 types of human malaria.

To date, demonstration of exoerythrocytic forms has depended largely upon examination of smears and sections of tissues. Recently Hawking[5,6] has shown that the exoerythrocytic forms of avian malarial parasites are readily grown in tissue cultures of infected tissues. This has been confirmed for *P. gallinaceum* by Haas,[7] Zuckerman[8] and by the present author.[9] Since methods of tissue culture allow for multiplication of the histotropic forms of the parasites, this technic might prove superior to the use of smears or sections in the search for mammalian exoerythrocytic forms. Accordingly we have made tissue cultures of bone marrow from humans infected with sporozoites of *P. vivax* or *P. falciparum* both before and after parasites

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1 Huff, C. G., and Bloom, W., *J. Infect. Dis.*, 1935, **57**, 315.

2 Porter, R. J., and Huff, C. G., *Am. J. Trop. Med.*, 1940, **20**, 869.

3 Brug, S. L., *Nederl. tijdschr. v. geneesk*, 1941, **84**, 2745.

4 Raffaele, G., *Trop. Dis. Bull.*, 1946, **43**, 1016 (abstract).

5 Hawking, F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1945, **39**, 245.

6 Hawking, F., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1946, **40**, 183.

7 Haas, V. H., personal communication, 1946.

8 Zuckerman, A., *J. Infect. Dis.*, 1946, **79**, 1.

9 Dubin, I. N., in preparation.

appeared in the blood.†

As a preliminary measure the technic was tested by cultivating the exoerythrocytic forms of *P. gallinaceum* from infected chick embryos.‡ These forms grew readily from spleen and liver as well as from brain and lung.[9] Hawking,[5] working with 10-day-old chicks, was unable to grow the parasites from the latter 2 tissues. Our difference in results is probably due to the greater ease with which embryonic tissues are cultivated as well as to the greater number of parasites in the embryonic tissues, as has been shown by Zuckerman.[8]

Methods and Materials. Sternal bone marrow punctures were made 4 to 6 days after the initial biting by infected mosquitoes, as well as 7 to 10 days after the development of parasitemia. The specimen was heparinized and centrifugalized. Tissue cultures were made from the fatty layer and from the buffy coat after it had been clotted with chick embryo extract. The cultures were made in bottomless Carrel flasks to which coverslips had been cemented.[10] The tissue was embedded in human plasma alone or in human plasma reinforced with chicken plasma. A drop of embryo extract was added to facilitate clotting. The fluid medium consisted of 25% human serum in Tyrode's fluid. The cultures were allowed to grow for 4 to 8 days at 37°-38°C. They were then fixed with formalin-Zenker fluid, stained with Maximow's stain, and mounted in clarite.[10]

Results. After negative results in 5 patients, 2 forms were found which bear resemblance to the exoerythrocytic forms of *P. gallinaceum.* These bodies were found in a 4-day-old culture of bone marrow taken 6 days after the patient had been bitten by mosquitoes infected with *P. vivax.* Five days after the sternal puncture was done parasites were demonstrated for the first time in the patient's blood.

Bodies found in tissue culture of human bone marrow from patient bitten by mosquitoes infected with *P. vivax.*

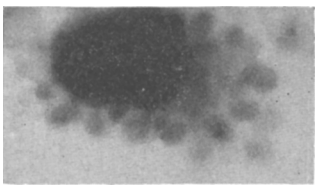
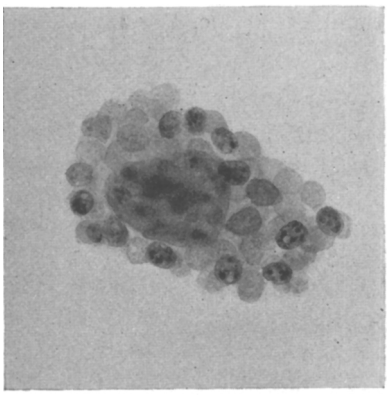


FIG. 1.
Photomicrograph of Form 1. Note the oval bodies surrounding the central nuclear mass. × 1600.



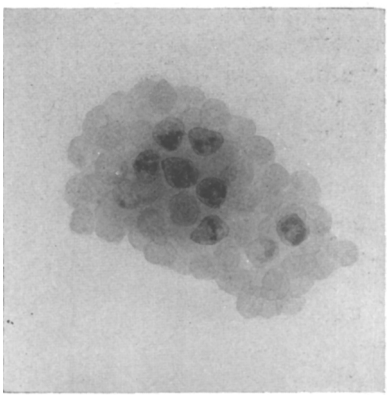


FIG. 2 AND 3.
Drawings of Form 1 at 2 different levels to show how nucleus is completely surrounded by oval bodies. × 1950.

The structures which we take to be exoerythrocytic forms of *P. vivax* are shown in Fig. 1 to 4. They were found in the immediate

† The patients were neurosyphilitics receiving malarial treatment on the Neuropsychiatric Service of the Gailor Memorial Hospital, Memphis. The author is indebteed to Drs. T. S. Hill, Henry Packer, and Y. T. Wong for the use of the clinical material.

‡ This material kindly supplied by Lt. Col. V. H. Haas, Office of Malarial Investigation, United States Public Health Service, Memphis.

10 Zuckerman, A., *J. Inf. Dis.*, 1945, **77**, 28.

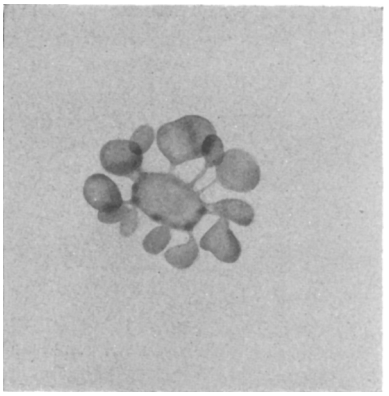


FIG. 4.
Drawing of Form 2. × 1800.

neighborhood of the implants. Form 1 (Fig. 1, 2, 3) shows a central nucleus surrounded by about 50 oval and round bodies. These small bodies have a sharp outline and an inner structure made up of vacuoles, wide pale-staining areas, and small dark granules. These bodies appear to be in the cytoplasm of a large macrophage. They resemble the larger merozoites described by Huff and Coulston.[11] Form 1 measured 21 by 12 μ.

Form 2 (Fig. 4) had a rosette appearance and showed a central core surrounded by radiating triangular or oval bodies. It measured 12 μ in diameter. It suggests a cluster of segmenters with a central core.

Discussion. These findings must be judged conservatively since (1) the forms found to date are so few, and (2) the criteria are purely morphological. The objection may be raised that in cultures of bone marrow degenerating cells are present which may be a source of error. The 2 bodies described above, however, are quite unlike the various forms taken by the degenerating cells. Moreover, their sharply-defined structures are suggestive of well-preserved animate bodies rather than of disintegrating cells. Furthermore, they resemble stages of exoerythrocytic forms in avian malaria.

Tissue culture studies are being continued in a search for additional forms in both human and simian malaria.

Addendum. After this article was submitted for publication there appeared a paper by Coulston and Huff[12] describing the exoerythrocytic forms of *Plasmodium relictum.* In the colored plates accompanying their paper are depicted forms which are similar to the 2 structures seen in the human bone-marrow. The oval bodies of the human Form 1 closely resemble the merozoites of *P. relictum* in Figs. 4, 7 and 13, while Form 2 is remarkably like the segmenter with a central core shown in Fig. 11.

11 Huff, C. G., and Coulston, F., *J. Infect. Dis.*, 1944, **75**, 231.

12 Coulston, F., and Huff, C. G., *J. Infect. Dis.*, 1947, **80**, 209.

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Tuberculostatic Action of Two Derivatives of the Alicyclic Compound, 3-n-Amyl-Cyclopentane-Carboxylic Acid.

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In an earlier publication it was shown that the sodium salts of certain synthetic alicyclic acids had tuberculostatic action.[1] Among the compounds tested sodium 3-n-amyl-cyclopentane-carboxylate (referred to as compound "C") was found to be among the most effective, 10 mg % being sufficient to give complete inhibition of growth of the tubercle bacillus (Strain A27) in Kirchner's medium. When the tuberculostatic action of this compound was tested by the chick chorio-allantoic technic,[2] it was found that 0.5 mg of the compound in a suspension containing

1 Emmart, E. W., *Am. Rev. Tuberc.*, 1946, **53**, 83.

2 Emmart, E. W., and Smith, M. I., *Am. Rev. Tuberc.*, 1943, **47**, 426.