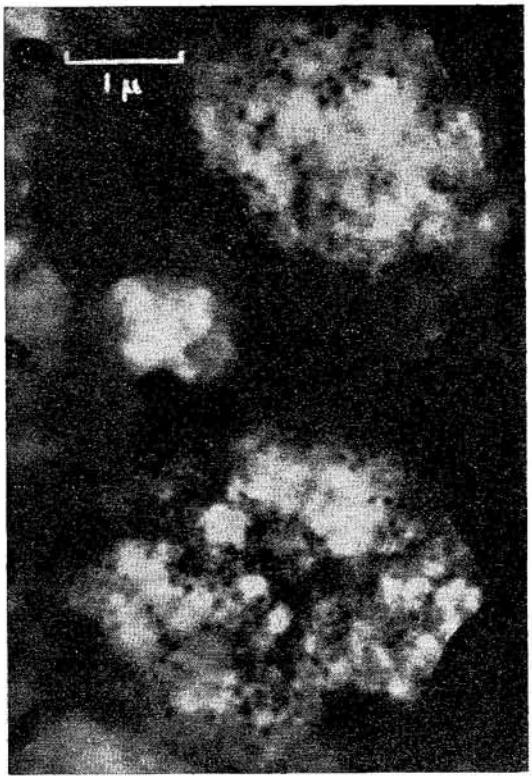

Fig. 2. Two vacuoles from another Rous ascites cell prepared as that of Fig. 1, and showing the virus-like particles at higher magnification. Electron micrograph, × 15,000

change is from the round form of the free dispersed single cells to a spindle form, and an early stage in the transformation consists of the cells spreading widely and becoming extremely thin. Ascites tumour cells spread thinly in this way are very suitable for electron microscopy ; they can be fixed over osmium vapour and transferred easily to electron microscope specimen grids with samples of the 'Formvar' to which they adhere[2].

This method avoids the labour and uncertainty of the tissue culture technique for preparing cells for electron microscopy[2], allows the cells to be prepared in large numbers and makes it unnecessary to keep them for many hours in an unnatural *in vitro* environment. It has been applied to the single dispersed tumour cells of an ascites form of the Rous No. 1 fowl sarcoma[4], in which all, or at the worst almost all, the cells flatten out under the conditions used. Thus, preparations made in this way avoid the problems of sampling inherent in the examination of ultra-thin sections of Rous sarcoma material.

Some 12,000 well-preserved Rous ascites cells from seven different tumours have been examined in the electron microscope. In some of the cells from four of these tumours, virus-like particles slightly less than 0·1μ in diameter have been observed ; in the other three tumours, no cells with particles were found, although about two thousand cells were examined from each. When cells with particles have been present, their incidence has varied from tumour to tumour, ranging from less than 1 in 2,000 cells to 1 in 50 cells ; it has, however, been constant for all the preparations made from any one tumour, thus arguing against a chance artefact being responsible for the appearances in question. The particles have always been found grouped together in one or more of the vacuoles common in this type of cell (Figs. 1 and 2) and are readily seen by phase-contrast microscopy when the cells are alive. No particles have been observed free in the cytoplasm of the cells of the present series, although cytoplasmic structure is well preserved by the technique used (Fig. 1).

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Role of 5-Hydroxytryptamine in Mental Diseases and its Antagonism to Lysergic Acid Derivatives

The possibility that 5-hydroxytryptamine plays a part in abnormal mental processes has been discussed by Gaddum[1] and by Woolley and Shaw[2]. The hypothesis is based on the findings that this substance is a natural constituent of nervous tissue, particularly of the brain[3], and that certain of its actions are antagonized by lysergic acid diethylamide[4], a drug which on oral application and in doses of only 0·5–1·0 μgm./kgm. produces pronounced psychic disturbances in normal human beings[5]. According to this hypothesis, the effects of lysergic acid diethylamide result from its antagonistic effect on the 5-hydroxytryptamine in the brain.

In the course of screening derivatives of lysergic acid diethylamide for 5-hydroxytryptamine-blocking effects, we have found in 2-brom-*d*-lysergic acid diethylamide, coded *BOL* 148, a compound which, like lysergic acid diethylamide, strongly antagonizes certain effects of 5-hydroxytryptamine, but unlike lysergic acid diethylamide does not produce abnormal psychic disturbances. The introduction of one bromine atom into the molecule of lysergic acid diethylamide thus completely alters its activity ; it loses the characteristic property of producing psychic disturbances in man but retains the strong anti-5-hydroxytryptamine action.

When comparing the central and peripheral effects of lysergic acid diethylamide with those of *BOL* 148, pronounced differences were found[6]. In normal and waltzing mice, lysergic acid diethylamide causes excitation, whereas *BOL* 148 has a sedative effect. In cats, small doses of the former cause a fall in arterial blood pressure and bradycardia, whereas the latter, tested in doses up to 1 mgm./kgm., was practically inactive. Again, the former has a strong stimulating effect on the rabbit uterus *in situ*, whereas the latter is practically inactive. We have also tested *BOL* 148 on ourselves and on some of our laboratory staff. It never produced signs of mental or psychic disturbances such as we regularly observed after lysergic acid diethylamide, even when used in doses twenty times as great. There was only a certain sedative action, consisting in a feeling of general fatigue and sometimes slight nausea.

In spite of the fact that the pharmacological effects of *BOL* 148 differ so completely from those of lysergic

acid diethylamide in animals as well as in humans, it was found to share with the latter the strong anti-5-hydroxytryptamine action. Erspamer[7] had shown that lysergic acid diethylamide is a strong antagonist to 5-hydroxytryptamine on the isolated rat uterus. On this preparation and on the isolated perfused kidney of the rat, *BOL* 148 was found to be an even stronger antagonist, being about one and a half times as active. In other tests *BOL* 148 was found to have approximately the same anti-5-hydroxytryptamine action as lysergic acid diethylamide, for example, in antagonizing the effects of intra-aortic injections of 5-hydroxytryptamine on the arterial blood pressure and the renal and mesenteric blood flow of the cat, in blocking the bronchoconstrictor effect of 5-hydroxytryptamine and in antagonizing its potentiating effect on barbiturates. The anti-5-hydroxytryptamine effect of *BOL* 148 is highly specific; in doses which fully antagonized the action of 5-hydroxytryptamine, *BOL* 148 showed no signs of an antihistamine, antiadrenaline or anti-acetylcholine effect.

As the hypothesis of the cerebral functions of 5-hydroxytryptamine is based to a great extent on the fact that lysergic acid diethylamide is a strong antagonist of 5-hydroxytryptamine, the present finding that *BOL* 148 is as active as lysergic acid diethylamide in antagonizing 5-hydroxytryptamine but produces none of the mental disturbances makes it necessary to reconsider this hypothesis. It cannot be argued that *BOL* 148 lacks cerebral actions because it does not penetrate into the brain tissue; the sedative action it produces is a central effect. In addition, after an injection, *BOL* 148 could be detected in the same indirect way as lysergic acid diethylamide in extracts of the brain, when tested for anti-5-hydroxytryptamine activity. Brain extracts of mice previously injected with either *BOL* 148 or lysergic acid diethylamide exerted anti-5-hydroxytryptamine activity[8].

Our results with *BOL* 148 thus make it difficult to correlate the psychic effects of lysergic acid diethylamide with its anti-5-hydroxytryptamine property. At present, we are not justified in assuming a causal relationship between these two properties of lysergic acid diethylamide, although it may eventually be found that the 5-hydroxytryptamine in the brain is involved in the central actions of lysergic acid diethylamide. The mere fact of a pharmacological antagonism between lysergic acid diethylamide and 5-hydroxytryptamine, however, no longer provides evidence for the hypothesis that inhibition of the latter in the brain is the cause of the mental disturbances.

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Transfer of Proteins from the Yolk to the Chick Embryo

The transfer of antibodies from the mother to the fœtus in certain mammals, and from the hen to the egg yolk and thence to the embryo in birds, is well established. Whether proteins other than antibodies are thus transferred is still open to question. Despite our lack of knowledge on this topic, it is generally thought, as Buxton[1] recently pointed out, that proteins pass from the blood or tissues of the hen as hydrolytic products which are reassembled into proteins within the ovum. Because of the proteolytic activity of the yolk sac and its contents, a similar mechanism is supposed to account for the passage of protein from the yolk into the circulation of the embryo.

We found recently[2] that, after intravenous injection into the hen, bovine γ-globulin and crystalline albumin, and whole lobster and rat sera are readily detected in the yolk by serological means. Further study of the bovine serum fractions in the yolk showed that they retained their respective electrophoretic mobilities on filter paper and could be displaced like the original proteins by ultracentrifugation. The bovine proteins deposited in the yolk do not interfere with development, since we have reared a number of the chicks to maturity and normal reproduction.

The experiments have now been extended to the passage of proteins from the yolk sac into the embryo. 0·3 or 1·0 ml. of 5 per cent solutions of Armour's bovine γ-globulin and crystalline serum albumin were injected into the yolk sacs of chick embryos varying in age from three to nineteen incubation days. Embryos older than six days were bled from the vitelline or allantoic arteries after various intervals of time; younger embryos were extracted *in toto* with 0·9 per cent saline. Rabbit antisera previously shown not to react in precipitin tests with rat, human and embryonic and adult chicken sera were used to detect bovine protein activity in the embryonic sera and body extracts. Fig. 1 shows the results obtained with γ-globulin. Fewer experiments have been done with albumin, but positive tests at consistently lower titres have been obtained.

Bovine γ-globulin was not detectable in sera or extracts prior to eight days of incubation. In the period between ten and fifteen days incubation, both

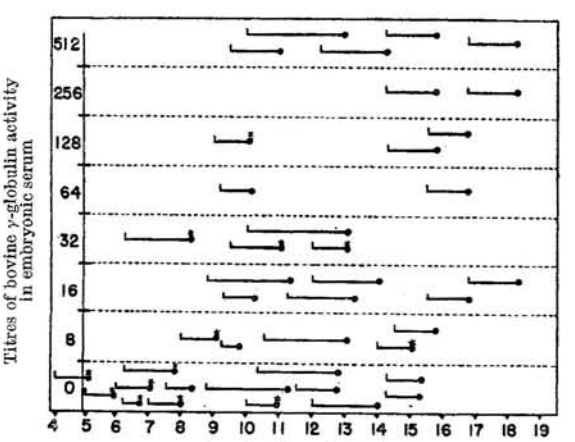

Fig. 1. Short vertical lines indicate when bovine γ-globulin was injected into the yolk sac. Black circles show when embryos were bled or extracted. * indicates pools of two or more embryos