of 'mestizos', that is, belonging to crosses with whites. (The total number of individuals *known* to be of *Ona*, *Yámana* and *Alakaluf* extraction, that is, 'Indians' *and* 'mestizos', as classified above, is, according to our information received from different sources, about 40 *Onas*, 60 *Yámanas* and 80–100 *Alakalufs*. The last figures are the least accurate because the *Alakalufs* wander in their canoes about the channels.) There were in the category of 'Indians' 5 *Onas*, 20 *Yámanas* and 9 *Alakalufs* or crosses between these : *all the 34 individuals were without any exception of group O*. On the other hand, in the category of 'mestizos' there were 43 individuals, of which only 24 were group *O*, and *all* the 19 mentioned belonging to *A*, *B* and *AB*. This sorting was possible thanks to the help of the Indians themselves, who have a fairly high level of education, and of the civil and medical authorities. So we were able to trace in most of these 19 cases the white source of the respective blood factor.

It is evident from our findings that the authentic Indians of the Tierra del Fuegan tribes of *Onas*, *Yámanas* and *Alakalufs* are all of group *O*, and that the presence of individuals of groups *A*, *B* and *AB* in these tribes is due to miscegenation with whites of Chilean, Argentine, Spanish or British extraction.

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[1] Rahm, G., *Rev. Instit. Bacteriol. de Chile*, 18 (1931).
[2] Lipschutz, A., "El Indoamericanismo y el Problema Racial en las Américas", 2nd edit. (Nascimento, Santiago de Chile, 1944).

Determination of Dienoestrol in Biological Samples

THE widespread clinical and veterinary use of the synthetic œstrogens has made it desirable to be able to assay these substances in biological samples, but until now no practical chemical method has been reported, although Dingemanse[1] and Malpress[2] have published methods applicable to non-biological samples.

A method for the determination of dienœstrol in biological material has been developed here, depending on the fact that dienœstrol combines with maleic anhydride to form a bicarbonate-soluble adduct.

Method. After acidifying, the sample is continuously extracted for 24 hours with benzene, the benzene extract concentrated and exhaustively extracted with aqueous sodium bicarbonate or carbonate. The aqueous layers are discarded. Excess maleic anhydride is then added, and after standing, the excess removed by water extraction. The dienœstrol maleic anhydride adduct is recovered by extraction with aqueous sodium bicarbonate, acidification of the latter, followed by ether extraction. Removal of the ether gives the free dicarboxylic acid of the adduct which may be weighed and then titrated against standard alkali, thus giving a value for the original amount of dienœstrol present.

In urine, recoveries of 60–80 per cent of added dienœstrol are obtained down to 1–2 mgm. per litre. Below these concentrations results tend to be high, due to the fact that there is a small blank reading with most urines so far tested.

It is probable that the method can be rendered more sensitive by applying a colour reaction to the adduct.

Further work is proceeding on the method, and detailed results will be published elsewhere.

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[1] Dingemanse, *Act. Brev. Neer.*, **9**, 118 (1939).
[2] Malpress, F. H., *Biochem. J.*, **39**, 95 (1945).

Folic Acid in the Nutrition of Certain Insects

WE have recently shown[1] that the caterpillars of the flour moth, *Ephestia kuehniella*, grow slowly on a purified diet in which all the known vitamins of the B-complex are provided in pure substance. The length of the larval stage is about 7–8 weeks, as compared with 5–6 weeks on a diet which contains yeast or yeast extract. A preparation of dried grass juice (Cerophyl Laboratories) was found to be an excellent source of the missing factor which, in its relations to charcoal, seemed to behave like folic acid[2]. It was adsorbed on norrite at *p*H 3 and was contained in the eluate, but not in the filtrate. We later obtained synthetic folic acid (Lederle Laboratories)[3] and established that this is the missing factor. On a purified diet, with folic acid, most of the larvæ pupated after 5–6 weeks; whereas without folic acid they mostly died and none pupated before the eighth week. The minimal optimal requirements seem to be about 2 μgm./gm. of the dry diet, that is, of the order of the riboflavin requirements of another insect, *Tribolium confusum*. We have also some evidence that crystalline vitamin B_c (Parke Davis and Co.)[4] has a similar, if not identical, effect as folic acid.

Folic acid is also of great importance in the nutrition of the mealworm, *Tenebrio molitor*. On a purified diet, which in addition to the known B vitamins also contains a liver charcoal filtrate, which by the nature of its treatment would be expected to contain little or no folic acid[5], growth is slow, and the mortality increased, while addition of synthetic folic acid has about the same effect as 1 per cent yeast, as seen in the accompanying table:

	After 9 weeks: Number surviving out of 40 larvæ	After 9 weeks: Average weight (mgm.)
1. Purified diet plus 1 per cent yeast	37	43
2. Purified diet plus liver charcoal filtrate	30	8·7
3. Purified diet plus liver charcoal filtrate plus folic acid (10 μgm./gm.)	40	38

These findings seemed to contrast with our earlier work with the flour beetle, *Tribolium confusum*, for which a mixture of the known vitamins of the B-complex in pure substances supported growth as well as yeast or an aqueous yeast extract[6]. On certain occasions recently it has been impossible for us to repeat this result, and all the evidence points to the fact that recent samples of casein, which had been highly purified for the purpose of assaying vitamins, were lacking in something which was contained in earlier samples. It is now clear that this deficiency is caused by a lack of folic acid. In an experiment, using 'Labco' casein, with folic acid added, the larval stage of the first eleven larvæ to pupate (out of twenty) lasted between 30 and 32 days, whereas without folic acid it lasted between 42 and 47 days.

Folic acid has also recently been reported to be of importance in the nutrition of mosquito larvæ, where it is necessary for pupation[7]. The authors of this investigation used, however, a concentrate of folic acid and not the pure substance.

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[1] Fraenkel, G., and Blewett, M., *J. Exp. Biol.*, in the press.
[2] Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, **141**, 521 (1941).
[3] Angier, R. B., *et al.*, *Science*, **102**, 227 (1945).
[4] Pfiffner, J. J., *et al.*, *Science*, **97**, 404 (1943).
[5] El Sadr, M. M., Macrae, T. F., and Work, C. E., *Biochem. J.*, **34**, 601 (1940).
[6] Fraenkel, G., and Blewett, M., *Biochem. J.*, **37**, 686 (1943).
[7] Goldberg, L., de Meillon, B., and Lavoipierre, M., *J. Exp. Biol.*, **21**, 90 (1945).

Gladiolic Acid : an Antifungal and Antibacterial Metabolic Product of *Penicillium gladioli* McCull and Thom

IT has been shown by Wilkins and Harris[1,2] that *Penicillium gladioli* produces an antibacterial substance when grown on liquid nutrient media. We have found that culture filtrates produced by this mould are toxic to fungi, the antifungal effect being more marked than the antibacterial effect. We have now succeeded in isolating the active substance in pure form from culture filtrates, yields as high as 300 mgm. per litre having been obtained, and have studied its biological and chemical properties.

The active material may be obtained by growing *P. gladioli* on shallow layers of Raulin–Thom medium (7·5 per cent dextrose) until an activity of 32 B.A. units per ml., as determined by the *Botrytis allii* spore germination assay[3], is reached. After removal of the mycelium, the culture filtrate is adjusted to *p*H 4·0 with hydrochloric acid and treated with activated charcoal (B.D.H.) at a rate of 5 gm. per litre. The charcoal is filtered off, dried at room temperature and extracted with ether. On evaporation of the ether, a brown or yellow pasty material is obtained.

This was purified by several recrystallizations from water and dried *in vacuo* over sulphuric acid. It crystallizes in long, colourless, silky needles, m.p. 160° C., containing no halogens, sulphur or nitrogen. It is optically inactive. Microanalyses (Weiler and Strauss): Found : C = 59·7, 59·75 per cent; H = 4·56, 4·74 per cent; $-OCH_3$ = 12·0, 11·3 per cent; mol. wt. (Rast.) = 212. $C_{11}H_{10}O_5$ requires : C = 59·4 per cent; H = 4·54 per cent; $-OCH_3$ = 14·0 per cent (for one $-OCH_3$); mol. wt. = 222·1. The compound is a monobasic acid (equivalent found by titration = 228, 207), and we propose to call it 'gladiolic acid'. A number of well-defined crystalline derivatives have been obtained, and its chemical properties are consistent with those that might be expected for a methoxy methyl-2-carboxyphenyl glyoxal.

Gladiolic acid gives an interesting colour reaction with strong ammonia solution. Initially a very deep green colour is produced but, after about 12 hours, this changes to red and finally, after several days, to orange.

Gladiolic acid shows some antibacterial activity. In nutrient broth at *p*H 7 it prevents growth of *Staphylococcus aureus* at 250 μgm./ml., but fails to prevent growth of *Salmonella typhi* or *Escherichia coli* at 500 μgm./ml. It is much more markedly fungistatic. As is usual with organic acids showing fungistatic activity, the activity is related to hydrogen ion concentration. At *p*H 3·5, where there is a higher proportion of undissociated molecules, there is a marked fungistatic effect, a concentration of 2 μgm./ml. preventing germination of *Botrytis allii* conidia; at *p*H 7, where the acid is largely dissociated, a concentration of 100 μgm./ml. is needed to prevent germination of *B. allii* conidia. Aqueous solutions of gladiolic acid in dilute buffer (McIlvaine's) retained their activity when stored for 10 days at 25° C. in a range of *p*H 3–8.