autopsied at the end of the two-month experimental period. Two of the total examined were found to be negative, but the remaining 35 mice were found to have *Toxoplasma* cysts in the brain. The inoculum has been examined microscopically. The only living material observed, apart from bacteria, were the oocysts of *Isospora* and the ova of *T. cati*. Nothing which could be related to *Toxoplasma* has, as yet, been observed.

On the basis of the facts presented, it appears that some form of *Toxoplasma* is capable of passing into the external environment in the faeces of infected cats. Under favourable conditions, this form can remain viable for at least twelve months and cause infection on ingestion by mice.

The results obtained are of a preliminary nature, but I feel that they are consistent enough to show that *Toxoplasma* can be isolated from faecal material by conventional techniques. At present it is impossible to say whether the infection induced by this material was transmitted in helminth ova or by some other means. Experiments now being conducted have been planned with the object of determining by which method the infection actually was transmitted.

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Resistance to Norleucine and Control of Methionine Synthesis in *Escherichia coli*

Norleucine appears to inhibit bacterial growth due to incorporation into proteins in place of methionine, as the analogue inhibits methionine incorporation but does not significantly reduce its synthesis[1]. It has been demonstrated[2] that resistance to amino-acid analogues in *Escherichia coli* is achieved frequently by over-synthesis of the amino-acid itself, and this appears to be the case with norleucine-resistant strains as some excrete a substance (presumably methionine) which stimulates the growth of sensitive strains in the presence of the analogue. Colonies of such resistant strains have haloes of growth around them when plated on sensitive strains in the presence of norleucine. A mutant (strain *P*-76-2) selected as a norleucine-resistant strain by this method had increased amounts of homocysteine methylase (compared to the parent strain *P*-76) when grown in minimal medium and the formation of the enzyme was only slightly reduced by growth with methionine[2,3]. It was proposed that strain *P*-76-2 failed to produce a repressor or formed a faulty one. The probable excretion of methionine and the high enzyme-level supported this idea. It was also possible that resistance to norleucine was due to decreased permeability to the analogue; failure of methionine to repress enzyme formation would then be due to a similar permeability restriction. The increased levels of homocysteine methylase on minimal medium are, however, not consistent with this view, and as methionine-requiring auxotrophs, isolated from strain *P*-76-2, grow well with small amounts of methionine there seems to be no restriction on the entry of this amino-acid. The control of methionine synthesis in strain *P*-76-2 has been further investigated to ascertain whether all the enzymes of the methionine-synthesis pathway are de-repressed and also to determine whether methionine exerts a feedback inhibition effect on the activity of the first enzyme as it does in other strains[4].

Homocysteine methylase has been assayed in strain *P*-76-2 and in two other norleucine-resistant strains. In strain *P*-76-2 (Table 1) growth with 2·5 mM methionine repressed enzyme formation by only 10 per cent compared to 88 per cent repression in strain *P*-76 (norleucine-sensitive) while in the other two strains (the growth of which was slightly inhibited by norleucine) repression of enzyme formation was 40 per cent compared to 86 per cent in the parent strain. Methionine synthesis in *E. coli* involves the functioning of three enzymes other than homocysteine methylase[5-7]. Homoserine *O*-transsuccinylase catalyses the synthesis of *O*-succinylhomoserine from homoserine and succinyl coenzyme *A*, cystathionine synthetase forms cystathionine from *O*-succinylhomoserine plus cysteine, while cystathionase produces homocysteine from cystathionine. Cystathionase and cystathionine synthetase were assayed in extracts from strain *P*-76-2 grown ± methionine; methionine (2·5 mM) did not significantly repress the formation of these enzymes (Table 2) although in other strains the formation of these enzymes is repressed by 80–90 per cent at this concentration of methionine. For the assay of homoserine *O*-transsuccinylase cystathionine-requiring mutants of strain *P*-76-2 were isolated. Extracts of mutants lacking cystathionine synthetase incorporated isotope from succinate labelled with carbon-14 into a product which behaved like *O*-succinylhomoserine on columns of 'Dowex-1' resin and on paper chromatograms. The formation of this homoserine *O*-transsuccinylase was not reduced by growth with methionine (2·5 mM), but methionine (5 mM) added to extracts prepared from organisms growth ± methionine exerted a marked inhibitory effect on enzyme activity. The extent of inhibition (90 per cent) was similar to that observed with norleucine-sensitive strains[4] (Table 3).

Thus in strain *P*-76-2 resistance to norleucine is associated with failure of methionine to repress any of the biosynthetic enzymes demonstrating that the mutation to resistance alters a single regulator site which in repressible strains controls the whole biosynthetic sequence presumably by the synthesis of a diffusible repressor. Although strain *P*-76-2 is non-repressible by methionine, the first enzyme of the biosynthetic pathway (homoserine *O*-transsuccinylase) is still sensitive to feedback inhibition of its activity. As the synthesis of *O*-succinylhomoserine by whole organisms is generally even more sensitive to feedback inhibition by methionine than synthesis by cell-free extracts[8], feedback inhibition ought to limit methionine excretion by strain *P*-76-2. It appears to do so, as I have been able to demonstrate only slight methionine excretion even after prolonged growth of

Table 1. Homocysteine Methylase Activity of Norleucine-resistant and Norleucine-sensitive Strains of *Escherichia coli*

Strain	Growth inhibition by DL-norleucine (mM) %	Homocysteine methylase (μm mole/mg dry wt./h) Organisms grown without methionine	Organisms grown plus methionine (2·5 mM)
P-76	99	260	30
P-76-2	15	570	530
R	96	350	50
*R*1	49	500	305
*R*2	38	610	390

Strains *R*1 and *R*2 are norleucine-resistant strains isolated from strain *R*.

Table 2. Effect of Methionine on the Formation of Cystathionase and Cystathionine Synthetase in *Escherichia coli* Strain *P*-76-2

DL-methionine added to the growth medium (mM)	Homocysteine methylase (μm mole/mg dry wt./h)	Cystathionase (μmole pyruvate/mg protein/h)	Cystathionine synthetase (μmole/mg protein/h)
0	740	1·9	1·8
2·5	690	2·0	2·1

Table 3. Effect of Methionine on the Formation and Activity of Homoserine *O*-Transsuccinylase

DL-methionine in growth medium (mM)	Homoserine *O*-transsuccinylase (counts/100 sec/mg protein/3 h) No methionine in incubation mixture	Methionine (5 mM) added to incubation mixture
0	11,250	650
2·5	11,500	1,450

strain *P*-76-2 in liquid medium while on solid medium excretion of methionine (detected by using norleucine-sensitive strains and methionine-requiring strains) occurred only after several days growth. Thus methionine over-synthesis appears to be limited by feedback inhibition in strain *P*-76-2 although the extra methionine formed due to the failure of enzyme repression is sufficient to overcome the inhibitory effect of norleucine.

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VIROLOGY

Dynamics of Antibody Production and of the Plasmocytic Reaction on Immunization with the Vaccinia Virus

THE mechanism of prolonged production of virus-neutralizing antibodies following single immunization with the vaccinia virus[1,2] is still obscure.

In order to follow the regularities of this process, non-inbred albino female rats weighing 150–200 g were used. The animals were immunized subcutaneously (the foot of the hind leg) with $9{\cdot}2 \times 10^6$ pock-forming units (titration on chick embryos[3]).

Decreasing quantities of the virus were found at the site of administration and in the popliteal (regional) lymphatic nodes during 96–120 h, and in the retroperitoneal and groin nodes during 48 h. No virus was isolated from the blood or internal organs.

Virus neutralizing antibodies (neutralization reaction on chick embryos[4]) appeared in the serum and lymphatic nodes on the 5th–7th day after immunization. Further experiments showed that the titres in the serum and homogenates of popliteal lymphatic nodes are kept at a constant level at least for 10 months (the observation term). In the retroperitoneal and groin nodes the antibody-level gradually decreased to zero during 1·5–2·0 months. In the homogenates of the spleen and axillary (distal) lymphatic nodes the antibodies did not always occur (in lower titres) and only during the first weeks after immunization. The antibody production presumably occurs mostly at the expense of regional lymphatic nodes. The reaction of the cells of the plasmatic series was examined in the prints of various lymphatic nodes stained with azur-eosin. In each preparation the cells were computed in 30 fields of vision. In parallel experiments homogenates of the same nodes were used for titration of the virus-neutralizing antibodies.

The results summarized in Table 1 illustrate the 7-month dynamics of antibody titres and the number of immature and mature plasma cells. In all experiments the dynamics of the number of blast-cells was parallel to the plasmocytic reaction.

It will appear from Table 1 that the intensity of the plasma cell reaction does not equal the number of antibodies in the homogenates of the lymphatic nodes and in the serum. The greatest number of plasma cells was recorded within the first 5–7 days following immunization when the titres of the antibodies did not always reach the highest level. At later dates, particularly in regional lymphatic nodes, a very moderate plasma cell reaction occurred while the antibody concentration did not decrease.

In repeated experiments similar results were obtained.

Thus, the prolonged and continuous process of antibody production caused by the vaccinia virus is not accompanied by an intense plasma cell reaction. The question arises: does the small number of plasma cells available suffice for the prolonged antibody production or is this effected by other cells?

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PSYCHIATRY

Failure to detect 3,4-Dimethoxyphenylethylamine and Bufotenine in the Urine from a Case of Periodic Catatonia

AMONG various biological active amines, bufotenine and 3,4-dimethoxyphenylethylamine have recently been given special attention in the psychiatric field because of their structural similarities to normal metabolites as well as their psychotomimetic properties. The presence of a bufotenine-like compound in 'schizophrenic' urine was first reported by Fischer *et al.*[1]. Recently Perry *et al.*[2] identified bufotenine in the normal urine from children with or without the administration of monoamine oxidase inhibitor, and Brune *et al.*[3] reported that they had confirmed the presence of bufotenine in 'schizophrenic' urine. Since Friedhoff *et al.*[4] reported the frequent occurrence of 3,4-dimethoxyphenylethylamine in 'schizophrenic' urine samples, the existence, the non-existence and/or the significance of this compound has been discussed[5–7] and is still open to discussion.

In this sort of work, however, a strict dietary control of the investigated subjects seems to be most essential. We have been investigating the amine metabolites in the urine of periodic catatonia cases under a strict dietary control, and some remarkable changes of phenolic amine metabolism during the psychotic phase have already been reported by one of us[8,9]. Therefore it also seemed

Table 1. NUMBER OF PLASMA CELLS AND TITRES OF VIRUS-NEUTRALIZING ANTIBODIES IN VARIOUS LYMPHATIC NODES OF IMMUNIZED RATS

Observation term	Antibody titre in the serum	Popliteal lymphatic nodes			Retroperitoneal lymphatic nodes			Groin lymphatic nodes			Axillary lymphatic nodes		
		No. of plasma cells		Antibody titre	No. of plasma cells		Antibody titre	No. of plasma cells		Antibody titre	No. of plasma cells		Antibody titre
		Immature	Mature		Immature	Mature		Immature	Mature		Immature	Mature	
5 days	1/40	235	18	1/40	132	4	1/40	68	3	0	21	16	0
7 days	1/320	340	11	1/160	26	14	1/160	3	4	1/160	26	4	1/20
1, 5 months	1/1,280	2	—	1/160	20	5	1/160	1	—	1/80	26	3	1/40
7 months	1/320	2	—	1/160	12	10	1/20	3	2	1/20	7	5	0

Note: (1) The high titre of antibodies in the serum taken after 1·5 month was a general feature of the given group of rats and does not signify a drop of the titre toward 7 months when another group was taken for the experiments.
(2) In the preparations from non-immune rats the number of plasma cells did not surpass 25–30 per field of vision.