Table 2. EFFECT OF D-TRYPTOPHAN ON RADIOACTIVE AMINO-ACID UPTAKE

Radio-active amino-acid *	Control c.p.m.	D-Tryptophan (50 μg/ml.) c.p.m.
L-tryptophan-^{14}C	321	180
L-proline-^{14}C	1,094	1,080
L-valine-^{14}C	1,185	1,053

The reaction mixture contained per ml.: 50 μg of dry weight of cells; 40 μg of chloramphenicol; 17 μmoles $MgCl_2$; 10 μmoles sodium formate; 10 μg L-amino-acid-^{14}C (10^4 c.p.m./μg); 25 μmoles *tris*; final *p*H 8·0[7]. After incubation for 1 min at 35° each reaction mixture was filtered on 'Millipore' filter *HA*. The filter was washed with 10 ml. of 0·9 per cent sodium chloride, dried and counted[7].

* All amino-acids; L-tryptophan, benzene ring uniformly labelled, prepared enzymatically[4]; L-proline, uniformly labelled; L-valine uniformly labelled; were adjusted to 10,000 c.p.m./μg with addition of carrier ^{12}C-amino-acid.

Even at the highest level employed (100 μg/ml.) growth of the *B*-8 cells was not completely inhibited by D-tryptophan. Morphologically, no shortening or elongation of the cells was observed in the concentration range of D-tryptophan (2·5–100 μg/ml.). The fact that *B*-8, a tryptophan auxotroph, is unable to grow in a minimal medium containing D-tryptophan (5–100 μg/ml.) indicates that D-tryptophan cannot substitute L-tryptophan for the growth of the auxotroph. The possibility was examined that D-tryptophan may inhibit bacterial growth by interfering with the uptake of L-tryptophan. To determine the effect of D-tryptophan on the uptake of L-tryptophan by the cells, washed bacteria were incubated in the minimal medium containing ^{14}C-L-tryptophan at 35°. In the presence of D-tryptophan uptake of radioactivity into the cells was significantly reduced (Table 2). Uptake of L-proline-^{14}C or L-valine-^{14}C was little affected in the presence of D-tryptophan. In the intracellular site, activation of L-tryptophan (L-tryptophanyl–adenosine monophosphate) and synthesis of L-tryptophanyl–RNA, which participate on protein synthesis, were not influenced in the presence of D-tryptophan[6].

A tryptophan transport system in *E. coli* reported by Boesi and DeMoss[7] and our findings that D-tryptophan inhibited uptake of L-tryptophan into a tryptophan auxotroph of *E. coli* suggest that a mechanism of the growth inhibition of a tryptophan–auxotroph, B-8, by D-tryptophan is the disturbance of L-tryptophan transport into the cells.

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Transformation of *Leptospira*

THE well-known pioneer experiments of Griffith[1], in 1928, proved the phenomenon of transformation in the *Pneumococcus*; the genetic characteristic transmitted was the virulence, of which the elaboration of the capsule is the phenotypic expression. Avery, MacLeod and MacCarthy[2] elucidated the cause: the genetic material, freed in solution, is sensitive to deoxyribonuclease; it is thus DNA. This pattern of 'transfer' has since been observed in other bacteria.

From our investigation on recombinations through transformation in *Leptospira* we used a strain of *Leptospira icterohaemorrhagiae* isolated in 1958 from a rat (*Rattus norvegicus*) captured in a coal-mine; the strain was named 'Cheratte' after its place of origin in the region of Liège. Cultivated in our laboratory, it has, as usual, quickly lost its pathogenic power.

Starting in February 1963 from the twelfth sub-culture, we succeeded through serial passages on guinea-pigs in restoring its virulence: it kills the animal within 6–8 days, showing the usual ictero-hæmorrhagic picture. The mixture of 1 ml. of virulent culture disrupted by successive freezing and thawing and 1 ml. of an initially non-virulent living culture has twice, in May 1963 and July 1964, caused the death of the guinea-pig together with icterus, numerous hæmorrhages and the presence in the organs of leptospires which can be re-inoculated in series. All these experiments were conducted with the necessary control experiments.

Besides this homotransformation, we succeeded in heterotransformation. The transfer principle came from the 'Cheratte' strain made virulent; the receiving microorganism was the 'Kantorowicz' strain, belonging to the serotype of group *Icterohaemorrhagiae*, for a long time designated as *A* or incomplete. This strain, isolated in 1931 at Amsterdam from the blood of a patient, lost its pathogenic power many years ago.

During the same experiment which allowed us, in July 1964, to succeed for the second time in our homotransformation, 1 ml. of the live culture of 'Kantorowicz', mixed with the same quantity of the killed culture of virulent 'Cheratte', resulted in the death of the guinea-pig on the eleventh day, with hæmorrhagic icterus. The 'Kantorowicz' strain thus made virulent remained so during several passages.

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CYTOLOGY

Distribution of Phthalocyanine Dye (Heliogen Blue *SBL*) in Ultrastructures of Vital Dyed Axons of the Crab *Carcinus maenas*

VITAL molecular disperse dyes are widely used in the investigation of permeability mechanisms and structural chemical changes in the cell[1]. The possibility of revealing such substances by means of the electron microscope was suggested by Isenberg[2] and later confirmed experimentally on preparations stained after the fixation[3,4]. Using an electron microscope Barrnett[5] revealed the azodye vitally formed in the cell as a result of a certain histochemical reaction.

The distribution of the acid substantive dye, heliogen blue *SBL* (HB) containing one copper atom, has been investigated with the aid of the electron microscope in vital-dyed axons of the crab, *Carcinus maenas*[6,7]. HB as observed on the film or in ultra-thin sections is characterized by a high electron density.

Axons were stained with 2 per cent HB solution diluted in artificial sea water without bivalent cations at *p*H 6·8 (phosphate buffer) for 2 h at 18°–20° C. Under such conditions diffusion equilibrium between the axon and the dye solution was reached in 1 h. The action potential of the axons in 2 per cent HB solution persisted for 3 days. Quantitative measurements of HB in crab's nerves prove that a half of the dye was dissolved or weakly bound, whereas the remaining portion was bound more strongly (Table 1).

For the electron-microscope investigation the stained and unstained axons were fixed in 2 per cent isotonic

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