saturating at 0.2 mM and 0.05 mM respectively. Approximately 2 mg of protein was used, in a final incubation volume of 0.5 ml.

Results and discussion. In the first experiment, EGTA (ethylene glycoltetraacetic acid) at various concentrations was preincubated at 37 °C for 5 min with the brain supernate to complex endogenous Ca^{++} which might normally be associated with the hydroxylase or which might have been liberated within the homogenate after redistribution from various compartments such as mitochondria; the reaction was then initiated by addition of 6 MPH_4. Curve A of figure 1 shows that EGTA has little effect below 5×10^{-5} M, but at this concentration enzymatic activity is markedly suppressed and then remains constant with increasing EGTA concentrations. The possibility that EGTA had deleterious effects in addition to complexing the Ca was tested by pre-incubating with EGTA, then adding an excess of Ca to give a final concentration of 2×10^{-3} M free Ca in all cases. Curve B shows that the reactivation of the enzyme by Ca varies after an initial exposure to EGTA, depending on the EGTA concentration. Reactivation by Ca is constant after exposure of up to 10^{-4} M EGTA, but is suboptimal after exposure to 10^{-3} M EGTA, suggesting that this higher concentration of chelating agent damages the enzymes. The effect of various concentrations of ionized Ca on enzymatic activity were then studied, by pre-incubating the brain extract with 10^{-4} M EGTA, followed by addition of Ca to yield the indicated ionized Ca-concentrations, as calculated by the method of Weber and Winecur[11]. Figure 2 shows that when the ionized Ca is adjusted to 10^{-8} M there is a reduction in activity from 70 ng of 5-hydroxytryptophan/mg protein seen with the native enzyme to 27 ng/mg at this low Ca^{++}-concentration. Increasing the free Ca-levels up to 10^{-5} M shows a trend of increased enzymatic activity which however does not become statistically significant until the Ca^{++}-concentration reaches 10^{-4} M. A sharp break in the curve is then seen, with marked increase in activity at 10^{-3} M free Ca, as observed by other workers[3,4]. The finding that the activity of the native enzyme is higher than that seen in presence of 10^{-4} Ca^{++} may merely reflect an activation of the enzyme by Ca liberated upon homogenization from mitochondria or other Ca-compartments, and as such would have little physiological significance.

The effects of other divalent metals were also studied in experiments (data not shown) in which brain supernate was pre-incubated with 10^{-4} EGTA, followed by addition of 2×10^{-4} M metal to yield approximately 10^{-4} free metal; hydroxylase activity was then assayed. Barium, zinc, strontium, magnesium and manganese had no effect on the EGTA-treated, Ca-depleted enzyme, but Fe^{++} was considerably more active than Ca. It has been suggested[12] that activation of tryptophan hydroxylase by Fe in vitro is due to the breakdown of H_2O_2 produced by the non-enzymatic oxidation of the reduced pterin cofactor; however, Fe could not be replaced by catalase in our experiments.

These experiments thus demonstrate that rat brain trytophan hydroxylase is not significantly activated by ionized Ca within the concentration range predicted from giant squid axon studies to be physiologically important, although activation becomes statistically significant at 10^{-4} M Ca^{++} and is very evident at higher metal concentrations, as shown by other workers[3,4]. It would thus seem that a direct activation of the hydroxylase by Ca^{++} has little physiological significance, and that other mechanisms must be sought to explain the regulation of this enzyme in vivo.

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Transcriptional effects of 5-bromo-2′-deoxyuridine in post-implantation mouse embryos

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Summary. Administration of 5-bromo-2′-deoxyuridine to pregnant mice on the 6th day p.c. brings about significant reduction in the incorporation of ^{3}H-uridine into RNA of 11-day-old embryos and causes elimination of a RNA species characteristic of that developmental stage.

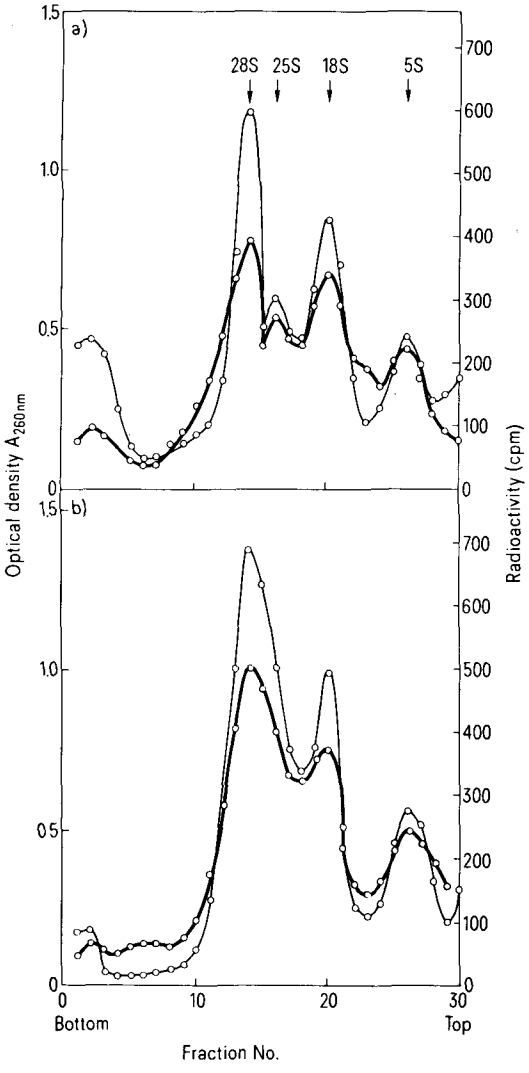
There is ample evidence to show that 5-bromo-2′-deoxyuridine (BUdR) inhibits differentiated functions of a variety of eukaryotic cells[3–6]. This effect has been purported to be mediated through the suppression of transcription of specific genes subsequent to the incorporation of the analogue into DNA[5–9]. Alternatively, it has been suggested that normal regulatory processes might be disrupted by thymine substitution of the target DNA by BUdR[8,10]. The former possibility is born out by the observations that the suppressive effects of BUdR on gene function are reversible by subjecting the substituted DNA to the dilution effects of growing the cells in BUdR-free medium[11–14]. The role of the latter course of events in determining the cellular responses to BUdR could be ascertained by studying the influence of the analogue exposure on the transcriptional process in a system, like developing embryos, in which interdependent sequential gene action is established. The present experiment has been designed on the basis of this concept.

Materials and methods. BALB/c$^+$ female mice $2^1/_2$–3 months of age, were mated with adult male mice of proved fertility and mating was confirmed by the observation of vaginal plug. On the 6th day postcoitum each pregnant mouse was given 2 injections of 500 µg of BUdR (Calbiochem.) dissolved in physiological saline via i.v. route. The interval between the injections was 7 h. On the 11th day of pregnancy, the animals were administered ^{3}H-uridine (C.E.N./S.C.K. Mol, Belgium; specific activity: 10 Ci/mmole) at 3.5 µCi/g body weight. 2 h after the injection, the mice were sacrificed and embryos collected. After washing in chilled saline, the embryos were pooled and used for RNA extraction by phenol-SDS method essentially as described by Brown and Littna[15].

Radioactivity of the RNA was determined by adding Instagel (Packard Inc.) to a known volume of the preparation and counting in a Packard liquid scintillation system. Counting time was so selected as to attain a

confidence level of 2 to 3%. RNA was analysed by sedimenting at 25,000 rpm on a 5–10% linear sucrose gradient in 0.01 M sodium acetate with 0.1 mM ethylenediamine-tetra acetate for 16 h at 4 °C in a Spinco SW 25 rotor. Gradient fractions were collected following passage through a recording spectrophotometer to trace the A_{260nm}. Radioactivity of the fractions was determined as described above. All the experiments were done in duplicate.

Results. Incorporation of radioactive uridine into RNA was significantly reduced in the embryos which were treated with BUdR on the 6th day post-coitum. The average radioactivity of the RNA extracted from the untreated embryos on the 11th day of pregnancy was 1540 cpm/100 µg while the RNA from the treated embryos of comparable age had only a radioactivity of 300 cpm/100 µg.



Sedimentation profiles of total RNA from control and BUdR treated embryos.

a Pregnant mice were administered ^{3}H-uridine (3.5 µCi/g body weight) on 11th day post coitum and sacrificed after 1 h. RNA was extracted from the embryos and analyzed on sucrose density gradients as described in 'materials and methods'.

b Pregnant mice were treated with BUdR on the 6th day of pregnancy and labelling, extraction and analysis of RNA, as described above, was done on 11th day of pregnancy. O–O–O–O optical density; –O–O–O radioactivity.

Figure 1a is the sedimentation profile in sucrose of total RNA from 11-day-old mouse embryos. The 25S RNA resolved in the gradient appears to be a molecular species specific to the 11-day-old mouse embryos. A significant consequence of BUdR treatment on the 6th day post-coitum was the complete disappearance of these characteristic molecules from the total RNA extract of the treated embryos on 11th day of embryogenesis (figure 1b).

Discussion. On the 6th day of pregnancy, the mouse embryo consists of only the three germ layers from which the entire organ system of the adult originates. As the whole embryo is mitotically active, BUdR exposure at this stage would result in an effective incorporation of the analogue into the DNA of a large proportion of the embryonic cells. On the 11th day of embryogenesis, when the transcription in the treated embryos was assessed, all the organ systems are fairly well differentiated and only their development is being continued.

Subsequent to BUdR treatment on the 6th day p.c. there is a sharp decline in the incorporation of ^{3}H-uridine into RNA of 11th day embryos. This might be due to a fall either in the rate of cellular uptake or utilization of ^{3}H-uridine or transcriptional rate of the embryonic cells. Fitmourice and Baker[16] who observed a reduction in the incorporation of ^{3}H-uridine into RNA of BUdR exposed sea urchin embryos suggested that the effect could be due to the molecules of BUdR competing with ^{3}H-uridine for entry into the cell's precursor pool. However, a parallel course of events is highly improbable in the present experiment as the presence of BUdR in the 11-day-old embryos is most unlikely following the treatment of the mother as early as 6th day of pregnancy. The observation, therefore suggests an interpretation involving active depression of RNA synthesis per se in the treated embryos. Such a transcriptional deficiency could conceivably be mediated through a general fall in the template efficiency of the embryonic DNA. However, as observed in microbial systems, BUdR could bring about alterations in the template activity of the DNA only if the molecule is extensively analogue-substituted[17]. And the DNA of the 11th day embryos cannot be expected to be modified to

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any significant extent, as the BUdR incorporated into the embryonic DNA on the day of treatment is expected to be subjected to the diluting effects of the subsequent divisions. Even if the modified form of the DNA existed in the treated embryos only for a limited period, it would affect the genes functional within that specified time span, as BUdR is demonstrated to disturb transcriptional process[8,18]. Such an effect, in turn, would influence every subsequent step in embryonic differentiation which, as described by Weintraub et al.[19], is temporally organized and 'involves the synchronous activity of a multitude of events'. Therefore, the decreased transcriptional activity observed in BUdR treated embryos could be the end result of a disturbance at one point in a system, the regulation of which rests upon interdependency of genes acting in sequence.

The disappearance of the 25S RNA which seems to be a normal compliment of the transcriptional products of 11-day-old embryos, in BUdR treated specimens also could be considered as a consequence of the disturbance described above. The absence of this RNA species in the experimental embryos might have been caused through the functional failure of the respective cistron or anomalous RNA processing which have been reported to occur following exposure of mammalian cells to other halogenated pyrimidines[20].

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Lethal effect of tetracycline on tsetse flies following damage to bacterioid symbionts

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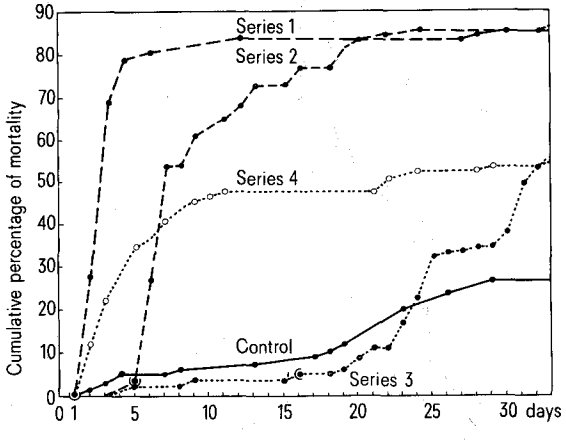
Summary. High mortality was observed in tsetse flies, Glossina morsitans morsitans, that had had a single blood meal on rabbits which had previously been administered tetracycline complex salts. The death of the flies was apparently effected by the killing of the fly symbionts and the destruction of the mycetomes of the gut. It is suggested that tetracycline complex salts in the food or drink of livestock may be tried for the control of tsetse flies.

Insects which are haematophagous throughout their lives, probably obtain essential vitamins from their symbiotic microorganisms[2]. It has been suggested that this dependence could be used for control by the elimination of the symbionts[3]. The bacterioid symbionts of Glossina are intracellular rodshaped organisms, located in a specialized cell group in the gut – the mycetome[4,5]. In the present report, tetracycline complexes were found to kill the symbionts and destroy the mycetomes of the gut of G. morsitans prior to the death of the flies. It is proposed that the flies died because of the accumulation of dead symbionts or direct poisonous effect of the tetracycline and not because of vitamin deficiency. Chlortetracycline has previously been used to obtain symbiont-free Glossina[6] without causing extensive death among the flies.



The cumulative mortality in Glossina morsitans series following a tetracycline containing rabbit-blood meal. The day of treatment is marked by a circle (same circle for series 1 and 4). Series 1–3 injected intraperitoneally; series 4 treated p.o.

Materials and methods. Glossina morsitans morsitans (Machado), obtained as pupae from the Tsetse Research Laboratory, Langford, Bristol, were maintained (up to 40 flies of mixed sexes to a cage) at 26 °C and 70% relative humidity. They were fed on rabbits on alternate days, starting on the first day after eclosion. Flies that refused to feed were discarded. The experiments consisted of a single tetracycline-containing blood meal given to series of Glossina 1 h after the drug had been administered to the rabbits. Other meals and the control feedings were on untreated rabbits. Tetracycline was injected i.p. or perorally to rabbits that had been starved for 24 h. The i.p. injections were given through a plastic tube that had been inserted through a small peritoneal incision, each time 24 h before injection. The tetracyclines used were the complex salt of oxytetracycline (Pfizer Italiana S. p. A., Italy) or chlortetracycline (American Cyanamid Co., Pearl River, N.Y.). 150 mg of the drug were mixed with 50 mg magnesium sulfate in 5 ml water, stirred slowly overnight at room temperature and injected i.p. at a dose of 50 mg tetracycline per kg rabbit body weight. 'Terra-

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