resulted. In these circumstances, however, the hypoglycaemic effect of hypoglycin was no greater, so it may be concluded that the mechanism of the effect is quite unrelated to inhibition of isovaleryl CoA dehydrogenase. A variety of other mechanisms have been proposed for hypoglycaemic action of hypoglycin and related compounds[6,13,14] and these remain for further investigation.

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Effect of olivomycin on the induced synthesis of tyrosine aminotransferase and tryptophan oxygenase in rat liver

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WHILE some inhibitors of RNA synthesis have been used to study the regulation of protein synthesis antibiotics of the olivomycin–mythramycin–chromomycin group have not been examined in this respect. The present paper shows that olivomycin inhibits almost completely the hydrocortisone induction of tyrosine aminotransferase (EC 2.6.1.5) and tryptophan oxygenase (EC 1.13.1.12), exerting only a slight effect on the substrate induction of tryptophan oxygenase. Part of these results has been reported previously.[1]

Female albino rats, weighing 150–180 g, were used throughout the experiments.

Olivomycin (a product of the Moscow plant for medical preparations No. 1) was dissolved in saline *ex tempore* and injected intraperitoneally (30 mg/kg body wt) 30 min prior to the injection of the inducers.

Hydrocortisone acetate, microcrystalline suspension (G. Richter, Budapest) was administered intraperitoneally, the dose being 100 mg/kg.

L-Tryptophan ("Serva") was dissolved in saline by the addition of 6 N NaOH. The solution was adjusted to pH 7·2–7·5 with hydrochloric acid and injected intraperitoneally at a dose of 1·0 g/kg body wt.

Abbreviations used: TAT, tyrosine aminotransferase; TO, tryptophan oxygenase; HC, hydrocortisone.

Adrenalectomy was performed translumbally under ether anaesthesia 5 days prior to the experiments. The adrenalectomized animals were given 1% NaCl solution to drink *ad lib.*

The animals were decapitated 4 hr after the administration of the inducer, between 12 a.m. and 1 p.m., i.e. at the time of the lowest circadian enzyme activity. The livers were chilled and homogenized in a Potter–Elvehjem glass–teflon homogenizer placed in an ice-bath.

The TO activity was assayed according to Knox *et al*,[2] after preliminary incubation with methemoglobin, tryptophan and ascorbate for activation of the latent forms of the enzyme. The livers were homogenized with 3 vol. of 0·14 M KCl, containing 0·002 M L-tryptophan and 0·1 M sodium phosphate, pH 7·0. The homogenates were centrifuged at 12,000 *g* for 20 min and the supernatant was used as enzyme preparation. The enzyme reaction was stopped by adding 24% metaphosphoric acid. After deproteinization and neutralization the kynurenine accumulation was estimated by the increase in A_{360} in an Opton–PMQ II spectrophotometer, using an extinction coefficient of $4{\cdot}53 \times 10^3$.

The TAT activity was measured by the method of Canellakis and Cohen,[3] as modified by Rosen *et al*.[4] The livers were homogenized with 9 vol. of 0·14 M KCl containing 0·005 N NaOH. The 12,000 *g* supernatant was used as enzyme preparation. The optical density of molybdenum blue was measured at 850 nm. An extinction coefficient of $4{\cdot}1 \times 10^3$ was used.[3]

The results obtained for both enzymes are expressed as units per gram wet weight, one unit being defined as the amount of enzyme yielding 1 μmole product after incubation for 1 hr at 37°. The data presented in this paper both for TAT and TO refer to the total enzyme activity after activation of the latent forms of these enzymes by preincubation with the cofactors of TAT and TO.

Statistical estimation of the results was completed by applying Student's *t*-test.

The effect of olivomycin on TAT and TO induction is shown in Tables 1 and 2.

TABLE 1. EFFECT OF OLIVOMYCIN ON THE HYDROCORTISONE INDUCTION OF TAT

	Treatment			
Adrenals	Untreated	Hydrocortisone	Olivomycin + hydrocortisone	Olivomycin
Adrenalectomized rats	32·9 ± 4·4* (13)	237·7 ± 8·0 (9)	77·3 ± 5·3 (10)	
Intact rats	46·9 ± 5·0 (18)	229·9 ± 11·0 (20)	56·7 ± 6·4 (10)	48·6 ± 4·8 (12)

Injection schedule and dosage, see Materials and Methods section. TAT activity is assayed at the fourth hr after the application of the hormone.

* Mean ± S.E.M. Number of animals in parentheses.

TABLE 2. EFFECT OF OLIVOMYCIN ON THE HYDROCORTISONE AND TRYPTOPHAN INDUCTION OF TO

Treatment						
Adrenals	Untreated	Hydrocortisone	Olivomycin + hydrocortisone	Tryptophan	Olivomycin + tryptophan	Olivomycin
Adrenalectomized rats	15·57 ± 1·64* (11)	81·90 ± 6·53 (9)	26·10 ± 3·27 (11)	35·24 ± 2·55 (12)	31·13 ± 12·06 (14)	—
Intact rats	24·09 ± 1·28 (15)	78·20 ± 4·22 (6)	27·83 ± 6·42 (9)	65·76 ± 5·91 (9)	43·80 ± 2·63 (20)	29·99 ± 1·93 (5)

Injection schedule and dosage, see Materials and Methods section. TO activity is determined at the fourth hr after the application of the tryptophan.

* Mean ± S.E.M. Number of animals in parentheses.

Adrenalectomy itself brought about a significant decrease in TAT and TO activity. These data corroborate previous findings[5,6]. In order to check the influence of the antibiotic on the enzyme level

in untreated rats with intact adrenals, we have determined the TAT and TO activity 4 hr after injection of olivomycin. The results obtained revealed that olivomycin by itself exerts no significant effect on TAT and TO activity ($P > 0.05$).

As can be expected, HC injection induced a many fold increase in TAT and TO activity at the fourth hr ($P < 0.001$), the induction being more pronounced in the adrenalectomized rats. Olivomycin, applied 30 min prior to the injection of HC, inhibited the hormonal induction of both enzymes by 80–90 per cent ($P < 0.001$).

Tryptophan injection caused a 2-to-3-fold increase of TO activity at the fourth hr after the application ($P < 0.001$). In this case, the induction was more pronounced in rats with intact adrenals. The latter observation can be explained by the combined action of both the inducer itself and the stress reaction which increases the plasma level of corticosterone in animals with intact adrenals. The effect of olivomycin administered 30 min before tryptophan induction differed from its effect on hydrocortisone induction. In adrenalectomized rats, olivomycin, administered 30 min before tryptophan, caused no significant inhibition. In intact rats the effect of olivomycin on tryptophan induction was analogous to that in adrenalectomized animals. The stronger inhibition of tryptophan induction in this case is conceivable if we take into consideration that this phenomenon is to a great extent due to the effect of the antibiotic on the stress induced rise of TO activity, mediated by the endogenous glucocorticoids.

Lajko[7] and Gauze *et al.*[8–10] have shown that olivomycin inhibits selectively RNA biosynthesis in bacteria and Ehrlich ascites carcinoma cells, whereas DNA and protein biosynthesis are affected negligibly. This effect has been ascribed to the inhibition of the RNA polymerase due to the formation of olivomycin–DNA complex.[8]

Olivomycin, mithramycin and chromomycin A_3 are related antibiotics which have a common (mithramycin and chromomycin A_3) or very similar (olivomycin) chromophore. They are the first antibiotics which have been shown to reproduce the effect of actinomycin D on RNA biosynthesis.[10,11] However, the nature of their interaction with DNA is clearly different from that of actinomycin[10,12] in that mithramycin and chromomycin do not intercalate their chromophore between two DNA base pairs in contrast to actinomycin D. That is why it was intriguing to explore the action of olivomycin on the induced synthesis of TAT and TO and to compare it with that of actinomycin D.

Greengard *et al.*[13–15] have demonstrated the different effect of actinomycin D and puromycin on HC and tryptophan induction of TO. They have established that puromycin inhibits both types of induction by more than 80 per cent, and inferred that both types of induction require new protein biosynthesis. The same authors have shown that actinomycin D, while inhibiting HC induction of TAT and TO by 90 per cent, has practically no effect on tryptophan induction (inhibition about 5 per cent). These results led them to the conclusion that while HC induction of TAT and TO is associated with new biosynthesis of RNA, the substrate induction of TO depends on other factors.

Our results show that olivomycin inhibits the HC induction of TAT and TO by more than 80 per cent. In contrast, tryptophan induction of TO is slightly affected by olivomycin in adrenalectomized rats. Therefore, olivomycin closely resembles actinomycin D in regard to its effect on the induced biosynthesis of TAT and TO. The only difference observed is quantitative, namely, the inhibition of substrate induction of TO by olivomycin is somewhat greater (about 20 per cent), as compared to that reported by Greengard *et al.* with actinomycin D (about 5 per cent). The reason for this difference is unclear and one could speculate that it is due to distinctions in chemical structure and nature of interaction with DNA.

In conclusion, olivomycin exhibits actinomycin D—like effect on the induced enzymes biosynthesis, inhibiting almost completely the hormonal induction of TAT and TO and only slightly influencing the substrate induction of TO, thus extending the possibilities of studying enzyme induction with inhibitors of RNA biosynthesis.

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A novel hypoglycemic compound*

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THE HYPOGLYCEMIC activity of 2-piperazino-4(3H)-quinazolinone monoacetate (referred to as Compound I) on rat and rabbit has been reported.[1,2] More recently the effect of Compound I has been studied in other species of normal animals and in diabetic rats and monkeys The results obtained are presented in this paper.

Albino rats of the Central Drug Research Institute colony (120–175 g) and the Charles–Foster strain (118–135 g); albino rabbits (1·2–1·5 kg) and guinea pigs (400–600 g) of the Central Drug Research Institute colony; mongrel dogs (6–11 kg) and rhesus monkeys (*Macaca mulatta*) (4–6 kg) were used in these experiments.

Compound I was administered orally as a solution in distilled water, in doses from 10 to 100 mg/kg body wt as shown in the tables. Control animals received an equal volume of the vehicle.

Alloxan, obtained from British Drug Houses, was administered to albino rats and monkeys. Administration of 1 to 4 units of Plain and PZ insulin per animal were needed to override the initial phase of hyperglycemia and acidosis for about 3 weeks. Albino rats after fasting had a blood sugar level greater than 400 mg per cent and monkeys had levels greater than 300 mg per cent. Only animals in this range were selected. Another group of albino rats were 95 per cent pancreatectomized by the method of Scow.[3] After recovery (28 days) they received an intramuscular injection of hydrocortisone (Calbiochem) (5 mg/rat/day) in 10 per cent ethyl–alcohol for 4–6 days to produce diabetes, characterized by a fasting blood sugar level greater than 200 mg per cent on 2 successive days.

The animals were fasted overnight, water being allowed *ad lib*. Blood from a vein was collected from the animals prior to administration of Compound I and later at various intervals. Blood sugar was estimated according to Somogyi's method as modified by Nelson.[4]

It can be seen from Table 1, that Compound I has a lowering effect on the blood sugar in albino rats, rabbits and dogs. However, the blood sugar of the monkey increases slightly. The blood sugar levels of guinea pigs were not altered.

From Table 2, it is clear that Compound I is capable of lowering the blood sugar of hydrocortisone treated pancreatectomized (max = 51 per cent) rats; whereas it has just a slight effect (max = 6 per cent) on alloxan-treated rats. Compound I lowers blood sugar levels of alloxan-treated monkeys, the maximum effect being 51 per cent, as is evident from Table 2. Thus the hypoglycemic character of Compound I has been confirmed in different species of normal and diabetic animals. It can lower blood sugar considerably when given orally to hydrocortisone-treated pancreatectomized albino rats

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An abstract of the paper consisting of a part of the data was accepted for presentation in the 7th Congress of the International Diabetes Federation at Buenos Aires in August 1970.