turnover in such a metabolic steady state did not show any difference between N and ADMX dogs both at neutral and at cold ambient temperature.

Discussion. Demedullation, as performed by the surgical procedure of Cannon et al.[7], did not induce any symptom of cortical deficiency. The animal remained in good health for the duration of the experiments, i.e., for a period of 3 to 12 months following surgery. Moreover, measurements of plasma cortisol showed that basal concentration, as well as the adrenocortical response to cold stress, were not impaired by adrenal demedullation. On the other hand, this type of surgery does not leave any adrenomedullary tissue, and the catecholamine secretory response to cold exposure is greatly reduced in adrenal-demedullated dogs[14,15]. The role of the adrenal medulla in thermogenesis is often difficult to evaluate, because of the lack of a satisfactory method for determination of the intensity of the cold stress. In the present experiments conducted on clipped dogs exposed to ambient temperatures of +25 °C and −21 °C, O_2 consumption increased to 4.4 times the BMR, and hypothermia was never observed. Similar results have been previously observed in adrenal-demedullated dogs[8,16] and cats[17]. In rats, the consequences of adrenal demedullation depend on the age (ref. in Himms-Hagen[6]): old rats are capable of shivering and piloerection, and they increase their O_2 uptake (just as controls do), while young ones do not maintain their body temperature when they are cold-exposed. During the present experiments, the body temperature of ADMX dogs decreased slightly, but significantly, at Ta = −21 °C. Therefore, it can be concluded that dogs deprived of their adrenal medulla are capable of making compensatory adjustments, in order to cope with their caloric requirements. However, their thermogenetic capacities are nevertheless reduced, and it is likely that intense cold stresses would disclose thermogenetic deficits due to adrenal demedullation which would induce hypothermia for a cold exposure of long duration.

A discussion of plasma glucose concentration should take into account several factors, such as the timing of the experiments and the length of previous fasting. Present data suggest that although the glucose pool, as determined from plasma glucose level, is somewhat smaller after adrenal demedullation, ADMX dogs can reach an equilibrium between production and utilization, which is not different from that of N dogs. Jarratt and Nowell[18] observed a decrease in glucose concentration in 24-h-fasted rats exposed to a +4 °C ambient temperature. This decrease was the same in controls and ADMX animals 2 h after the start of cold exposure, but was greater in ADMX animals than in controls 10 h after. On the other hand, adrenal demedullation can eliminate the hyperglycemia sometimes induced by intense cold exposure in short-fasted rats[19,20] or in dogs[14].

The tracer methodology employed in the present experiment allows an overall picture of glucose metabolism, but does not provide any insight into the sites of glucose release and uptake. However, with reference and analogy to data collected in man (*ref. in* Felig[21]), it is likely that, in 15 h-fasted dogs, the almost totality of glucose production has a hepatic origin, the renal contribution being negligible. At neutral or cold ambient temperature, plasma glucose concentrations remained fairly constant both in normal and ADMX dogs. Therefore, it is possible to consider that, under these experimental conditions, hepatic glucose output was almost equal to glucose utilization.

The present study shows that dogs deprived of adrenal medulla are able to increase glucose production and utilization in the same manner as normal dogs. However, it cannot be inferred from these results that, in normal dogs, circulating epinephrine has no effect on glucose irreversible production by the liver. Actually, as recently stated by Goodner[22], the regulation of hepatic glucose production probably reaches a high degree of redundancy and includes many systems, such as secretion of pancreatic glucagon, reduction of insulin secretion, and stimulation of the direct autonomic innervation of the hepatic parenchymal cells. One may postulate that, when present, as in normal dogs exposed to cold, adrenal medullary secretion affects glucose output and plasma concentration. However, when it is not present, these animals are capable of making other adjustments, in order to maintain their glucose homeostasis.

In conclusion, it appears from these experiments that the thermogenetic capacities and the glucose homeostasis are not clearly affected by adrenal demedullation in dogs submitted to a cold severe enough to rise the energy expenditure 4.4 times above the resting metabolic rate.

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An electrophoretic investigation of the binding of 3-^{14}C coumarin to rat serum proteins

N. B. Piller and L. H. Schmitt

Electron Microscope Unit, University of Adelaide, and Department of Genetics, University of Adelaide, G. P. O. Box 498, Adelaide (S. Australia 5001), 3 January 1977

Summary. The binding of coumarin to serum proteins of the rat has been demonstrated. Of the total bound coumarin (37% of injected dose), 36% was bound to the slow and fast α_1 globulins, 11% to the post albumins, 10% to globulin and 9% to albumin.

The binding of coumarin (5-6-benzo-α-pyrone) to purified serum and plasma albumins has been reported by Garten and Wosilait[1] and by O'Reilly[2]. The report of Garten and Wosilait[1] suggests ~40% of coumarin binds to bovine serum albumin in vitro. Bauer-Staeb and Niebes[3] reported a range of binding for the related O-β-hydroxyethyl derivatives ranging from 5% for the tetra-hydroxyethyl rutoside to 71% for rutin. They used human serum in its unpurified form.

Piller[4] reported the binding of coumarin to rat serum proteins. In vitro, over a dose range of 3.3–50 μg/ml binding remained constant at ~40% while in vivo over the

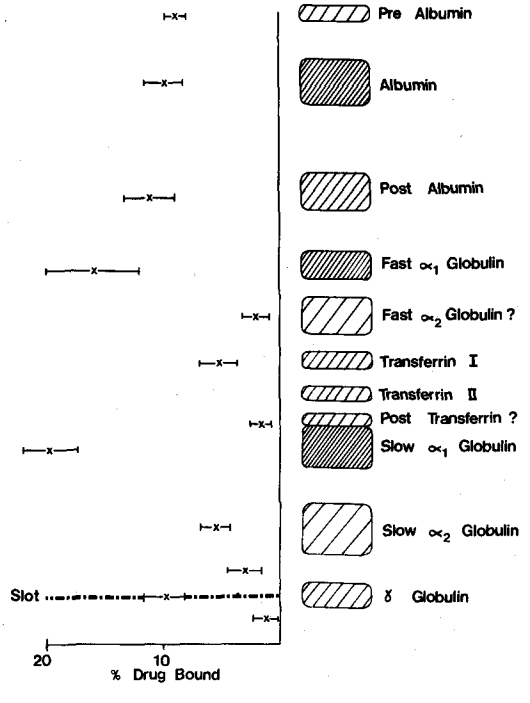
same dose range ~37% was in the bound form. This study did not include possible binding to protein fragments. There is no mention in the literature as to exactly what proteins coumarin can bind to. The only estimates available are those of Garten and Wosilait[1] who used a crystallized albumin preparation.

This experiment was designed to determine the binding of 3-^{14}C coumarin to normal rat serum proteins under in vivo conditions.

Materials and methods. 3-^{14}C coumarin, specific activity 5.5 mCi/m mol (37.5 μCi/mg was obtained from the Radiochemical Centre, Amersham, England). An analysis of radiochemical purity ascertained by thin layer chromatography on silica gel in a) benzene:ethanol (90:10), b) toluene:ethyl formate:formic acid (50:40:10) and c) benzene:chloroform 50:50 gave results of 99%, 99% and 98% purity respectively. An analysis of chemical purity showed the IR absorption spectrum to be identical to that of coumarin reference materials. (Information supplied by the Radiochemical Centre.)

3-^{14}C coumarin at a dose level of 25 mg/kg in a 2% solution of A.R. ethanol in physiological saline was injected i.v. into each of 5 female albino rats of the S.P.F. strain (average weight 200 g). After 15 min, blood was removed by heart puncture and allowed to clot. Blood plasma (8 μl) in duplicate was added to the slits of a 10-channel starch gel.

Separation of the plasma proteins was carried out by horizontal starch gel electrophoresis in a water cooled gel bed. The electrolyte buffer was 0.06 M lithium hydroxide, 0.229 M boric acid (pH 8.5), while the gel buffer was a mixture of the electrolyte buffer and a 0.079 M tris (hydroxymethyl) aminomethane, 0.007 M citric acid buffer in the ratio 1:5·4 (pH 8.5)[5]. Connaught hydrolysed starch was used at a concentration of 11.5%.



Schematic representation of the results of starch gel electrophoresis together with the suggested designation of protein zones (nomenclature derived from Beaton et al.[6]). The percentage of the total protein bound drug is shown for each band. Each estimation represents the mean of 5 duplicate determinations.

Electrophoresis was carried out for 4 h at 13 V/cm. The gel was then stained with amido black solution (3.7 g in 250 ml H_2O + 250 ml methanol + 50 ml glacial acetic acid). The gel was destained overnight in the same solution without amido black. An attempt was made to name the protein bands based on earlier results of starch gel electrophoresis of rat serum proteins[6].

For scintillation counting each of the duplicate gels were divided into 2-mm sections for 10 mm below the slot and for 60 mm above. This pattern was varied where necessary to accommodate the protein bands. Only the total activity of the whole bands are given. Each piece was finely razor-minced and placed in a plastic scintillation vial. To each, 1 ml Soluene (Packard) was added and this incubated at 60 °C for 2 h or until the gel dissolved. 10 ml of Insta Gel (Packard) was added, followed by shaking and counting in a liquid scintillation counter. All sections with a count which was not significantly different from the overall gel background were ignored.

Results and discussion. Previously[4], with i.v. administration of 3-^{14}C coumarin, approximately 37% of the injected dose bound to serum proteins. Garten and Wosilait[1] using purified bovine albumin presented evidence of a 40% binding of coumarin. Contrary to this the figure shows that only 9% of the total bound coumarin was bound to what was electrophoretically distinguishable as albumin. Since the total coumarin bound is approximately 37% of the injected dose this represents only 3.3% of the total coumarin. Such differences are however not surprizing since the benzopyrones in general and coumarin in particular have been frequently reported to exhibit wide species variation in their actions and properties[7].

The figure shows a high percentage of the coumarin becomes bound to what is called the slow and fast α_1 globulins[6] (19.6% and 15.8% respectively). Although there is little decisive information available, some evidence suggests that these globulins are of considerably lower mol.wt than albumin[8]. Post albumins, to which 11% of the coumarin is bound have a mol.wt ~100,000. γ globulin, to which 10% was bound has a mol.wt between 156,000 and 161,000. The only protein to which coumarin became bound (5.6%) which has a very high mol.wt is slow α_2 globulin[8]. Thus the greater proportion of coumarin which is bound is bound to relatively low mol.wt proteins.

Renkin et al.[9] report the small pore system (radius 40 Å – c.f. albumin 35.5 Å) to permit the exchange of low mol.wt solutes (free coumarin has a mol.wt of 146), although the permeability rapidly declines as the molecular size nears that of the pores. There seem to be 2 systems by which the larger mol.wt molecules are transported[9]. About one half occurs via turnover of endothelial vesicles (radius 250 Å) and one half by ultrafiltration through large gaps or pores with a radius in excess of 1600 Å.

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Certainly, the small pore system is important for the exchange of free coumarin and for that bound to the fast and slow α_1 globulins (mol.wt ~45,000) and explains the rapid entry of coumarin into most tissues[10]. The endothelial vesicle and large pore system will allow the entry, albeit slower, of coumarin bound to the larger macromolecules like α_2 globulin.

The mode of action of coumarin and related drugs is very complex[7,11] and while it seems that either a protein-coumarin type complex or just free coumarin could be responsible for macrophage activation[7,12–14], which results in increased protein lysis[15] through its intra and extra cellular digestion, we have yet to elucidate the exact importance of the free and bound coumarin.

It has frequently been reported[7] that in the initial 30 min after benzopyrone administration, there is the release of endogenous amines which result in the opening of additional numbers of endothelial junctions[7,16], and allow some extra protein (and protein bound coumarin) into the tissues. I must mention here that this effect is transient and the small additional protein inflow is more than compensated for by the later action, that of enhancing the lysis of all accumulated protein[7,17,18]. The effect of the drug in causing the opening of additional endothelial junctions does however allow extra protein bound drug into the tissues and into close proximity to the target cells.

Further work is currently in progress to ascertain the importance of drug protein binding in models of mild thermal oedema, acute and chronic lymphoedema and to relate this to the effectiveness of coumarin as an oedema reducing agent.

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The effect of L-Dopa on the spinal monosynaptic mass reflex

J. Geber and M. Dupelj

Department of Pharmacology and Department of Neurology, Medical Faculty, University of Zagreb, 41000 Zagreb (Yugoslavia), 10 January 1977

Summary. After i.v. injection of reserpine, the monosynaptic mass reflex (MSMR) is depressed in spinalized cats. However, the complete recovery of MSMR was obtained 30 min after L-Dopa application. Pimozide, a dopamine-receptor blocking agent, blocked this action of L-Dopa. It is presumed that dopaminergic receptors are involved in the action of L-Dopa on spinal MSMR.

Alterations in central catecholamine metabolism have been found to attend a number of neurological disorders. These changes have often been assumed to reflect altered nerve activity in neural systems containing these amines. Dopamine (DA) is a widely distributed, naturally occurring compound[1]. The highest level of DA has been found in the neostriatum in conjunction with the dopaminergic nigro-striatal pathway[2]. However, recent investigations have shown relative high concentrations of DA in the spinal cord[3–7]. Its role in the central nervous system, both as a precursor to noradrenaline (NA) and as a putative neurotransmitter, has been intensively studied[2,8]. The object of this study is to examine further the validity of this assumption by studying the effects of L-Dopa (L-3,4-dihydroxyphenylalanine) on spinal MSMR.

Materials and methods. Experiments were performed on 18 adult cats of both sex. Cats were anaesthetized with thiopental (30 mg/kg i.p.) and the spinal cord was transsected at C_1 segment. The animals were maintained in a slightly hyperventilated condition by respiratory pump. A dorsal laminectomy was performed from L_6 to S_1, and the cord was exposed and covered with warm mineral oil. The medial gastrocnemius plus soleus nerves (GS) were isolated for electrical stimulation. The GS was stimulated (5–10 V/0.1 msec 0.3 Hz) and its monosynaptic reflex recorded monophasically in the L_7 or S_1 ventral root. The resulting electrical activity was amplified and displayed according to conventional methods and recorded before and after i.v. injection of reserpine, L-Dopa and pimozide (diphenylbutyl piperidine).

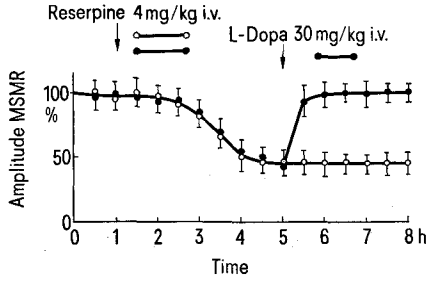

Fig. 1. Effect of reserpine and L-Dopa on monosynaptic mass reflex (MSMR). All values are graphed as percentage of the control value obtained just prior to drug injection. Each point represents the mean of 6 experiments (± SEM).

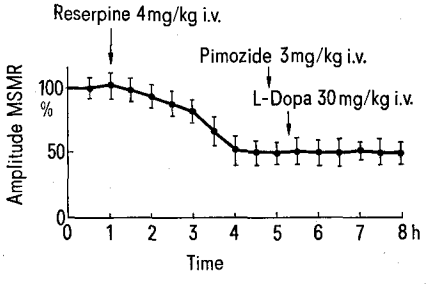

Fig. 2. The time course of pimozide antagonism of the effects of L-Dopa on monosynaptic mass reflex (MSMR). Each point represents the mean of 6 experiments (± SEM).