fluorescence in freeze-dried material has been reported[6] but no explanation has been proposed for this effect.

Our results indicate that in spite of the disadvantages of unfixed cryostat sections, these appear as the only suitable preparations for the localization of transplantation antigens in solid tissues by means of immunofluorescence.

Résumé. Plusieurs fixateurs produisent des effets délétères sur les antigènes de transplantation de la souris. La fixation à froid, la congélation-substitution et la cryodessication ne donnent pas de résultats satisfaisants.

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Effect of Blood Sampling Methodology on Plasma Levels of Corticosterone, Inorganic Phosphorus and Serum 5-Hydroxytryptamine Concentrations[1]

A wide variety of experimental factors including time of day[2,3] sex[2], environmental change[4-6] and level of anesthesia[7] have been reported to influence plasma corticosterone concentrations. In addition, it has been suggested that the methodology used in obtaining blood samples may also affect plasma levels of corticosterone[8]. Therefore, the present experiments were undertaken to explore the possible influence of blood sampling methodology on serum 5-hydroxytryptamine (serotonin) and plasma inorganic phosphorus as well as plasma corticosterone concentrations.

Materials and methods. Animals used in the present study were female Sprague-Dawley rats (Charles-River) that were housed 2 per cage for at least 3 weeks under conditions of controlled lighting (fluorescent illumination from 0400–1800) and temperature (24 ± 2°C). Purina laboratory chow and tap water were available ad libitum. 3 days prior to the experiment rats were transferred to individual cages.

In all experiments rats were taken individually from the animal quarters to the adjoining preparation room were they were subjected to one of the following methods for obtaining blood samples: Decapitation (DC), rats were rapidly decapitated (< 20 sec following initial handling) and 2 ml of trunk blood collected in a centrifuge tube to which 0.25 ml ascorbic acid had been added; the remaining trunk blood (3 ml) was collected in a heparinized centrifuge tube. Cardiac tap (CT), each rat was rapidly weighed and injected with sodium pentobarbital (35 mg/kg, i.p.). Exactly 10 min following pentobarbital injection, 5.0 to 6.0 ml of blood was withdrawn within 1 min from the heart into a 10 ml saline-rinsed syringe (1 inch 21 gauge needle). 2 ml of heart blood was then injected into a centrifuge tube to which 0.25 ml of ascorbic acid had been added; the remaining blood was deposited in a heparinized centrifuge tube. Jugular vein tap (JV), rats were rapidly anesthetized with ether, the external jugular vein exposed and 2 ml of blood collected in a saline-rinsed syringe (1 inch 21 gauge needle) following which 3.5 to 5.0 ml of blood was collected in a separate heparinized syringe; both samples were collected within 3 min following time of cage opening. In all cases, non-heparinized blood was mixed gently with 0.25 ml of ascorbic acid, centrifuged following clot formation and serum collected for serotonin determinations. Heparinized blood was centrifuged immediately and plasma collected for corticosterone and inorganic phosphorus determinations. Sampling periods, which began at 08.00 or 16.30 h of the same day, were approximately 90 min in duration.

Plasma levels of corticosterone and inorganic phosphorus were determined by the fluorometric method of GUILLEMIN et al.[9] and colorimetric method of FISKE and SUBBAROW[10] respectively. The fluorometric method of WEISSBACH et al.[11] was used to determine serum levels of serotonin. The amount of hemolysis was subjectively evaluated and correlated with the method of sampling blood by using an arbitrary scale of 0 = no hemolysis, + = slight hemolysis, ++ = considerable hemolysis and +++ = near maximal hemolysis. Statistical probabilities were derived from analysis of variance or Student's *t*-test.

Results. Analysis of variance revealed a significant difference between the corticosterone values of the 3 groups during morning but not afternoon experiments (Figure 1). Individual comparisons showed AM corticosterone values of the CT group to be higher ($P<0.05$) than those of DC or JV groups; DC and JV groups were not different. AM-PM differences ($P<0.01$) in plasma corticosterone were observed in DC and JV but not CT animals.

As summarized in Figure 2, plasma inorganic phosphorus was highest in DC rats and lowest in CT animals at both time points; concentrations in JV rats were intermediate and different ($P<0.01$) from CT and DC bled rats in the AM but not PM. AM-PM differences in inorganic phosphorus were not observed in any of the experimental groups.

As with the corticosterone and inorganic phosphorus, marked differences in serotonin concentration were observed (Figure 3). Individual comparisons indicated that serotonin values in JV rats were higher at both time points

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($P<0.01$) than those from CT or DC rats. No significant AM-PM differences were observed. Whereas, plasma from JV rats showed little or no hemolysis, plasma samples of DC and CT rats were markedly hemolyzed.

Discussion. The finding that corticosterone levels varied with the time of day was not unexpected since the experimental hours were time periods known to represent the high (PM) and low (AM) periods of the 24-h plasma corticosterone circadian rhythm[2,3]. It was surprising that AM-PM differences in corticosterone concentration were not observed in rats subjected to cardiac tap inasmuch as SCHEVING et al.[3] using cardiac tap as the method for collecting blood demonstrated a prominent circadian rhythm in plasma corticosterone. Absence of an AM-PM difference in corticosterone concentration in the CT group is consistent with the study of CRITCHLOW et al.[2] which demonstrated that pentobarbital (50 mg/kg) acting for 30 min suppressed AM-PM differences in plasma corticosterone. However, in the present study absence of the expected diurnal variation in plasma corticosterone in the CT group appeared to be the result of elevated AM corticosterone levels and not suppressed PM plasma corticosterone concentrations as in the study of CRITCHLOW et al.[2]. Although the explanation for these differences cannot be ascertained at present, the controversy regarding the effects of pentobarbital on plasma corticosterone levels is recognized. Had we employed a higher dosage of pentobarbital and/or waited a period of time greater than 10 min before collecting cardiac blood the results may have been different. However, the dosage (35 mg/kg) used in the present study is an accepted dose for experimental studies[12] and the 10 min latency was the average time for complete anesthesia. The observation that plasma inorganic phosphorus was highest in blood collected following rapid decapitation suggests that the methodology involved in CT and JV bleeding procedures may have resulted in suppressed inorganic phosphorus concentrations. Whether these differences in plasma levels of inorganic phosphorus reflect an effect of the stress or the anesthesia attendant with the CT and JV bleeding procedures are not known. Consistent with the latter alternative are data which indicate that plasma inorganic phosphorus concentrations are not affected by immobilization stress (unpublished observation). However, the increased serotonin concentrations noted in serum of rats bled via the jugular vein may reflect the use of ether anesthesia inasmuch as preliminary findings in this laboratory indicate that exposure to ether vapor results in rapid changes in serum levels of serotonin. The absence of AM-PM differences in inorganic phosphorus and serotonin concentrations was expected since the times selected for sampling in the present study represent periods of the day when circulating inorganic phosphorus[13] and serotonin[14] concentrations would be quite similar[13]. Although hemolysis may not markedly affect the analysis of corticosterone, inorganic phosphorus or serotonin, the effect of hemolysis on the analysis of other variables such as potassium and certain enzymes is well known. Thus, it was of considerable interest to find that non-hemolysed blood can be obtained routinely using the JV method for blood collecting. Although explanations for many of the

Effect of blood sampling methodology on plasma hemolysis

AM			PM		
CT	DC	JV	CT	DC	JV
+[a]	++	+	0	+	0
+	+	0	+	++	0
0	++	0	+	++	0
++	0	0	+	++	0
++	+++	0	++	+++	0
+	++	0	++	+++	0
+++	+++	0	+	+++	+

[a] 0, no hemolysis; +, slight hemolysis; ++, considerable hemolysis; +++, near or maximal hemolysis.

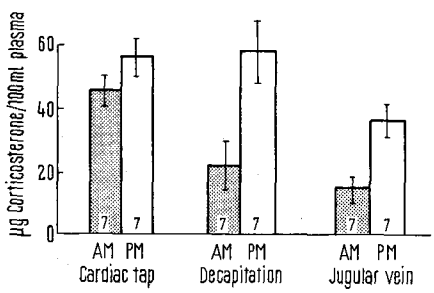

Fig. 1. Effect of methodology on plasma levels of corticosterone in female rats. In this and subsequent illustrations number of animals per group is indicated at the bases of the columns; vertical lines indicate standard error.

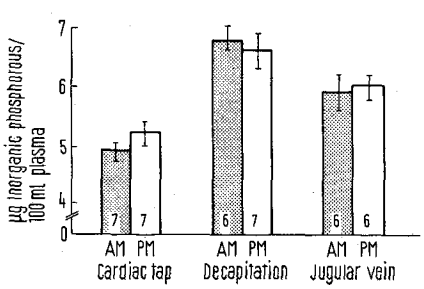

Fig. 2. Effect of methodology on plasma inorganic phosphorus concentrations in female rats.

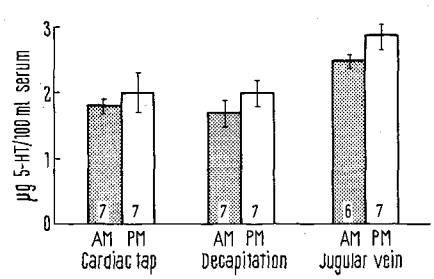

Fig. 3. Effect of methodology on serum levels of 5-hydroxytryptamine (serotonin) in female rats.

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above differences have not been determined, these data further document the influence that methodological procedures may have on experimental data.

Zusammenfassung. Nachweis, dass diverse konventionelle Methoden der Blutgewinnung (Herzpunktion, Dekapitieren, Punktion der Jugularvene) zu unterschiedlichen absoluten Werten von Serum Corticosteron, anorganischem Phosphat und 5-Hydroxytryptamin führen, wobei auch eine unterschiedliche Hämolyse resultierte.

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The Effect of Pentagastrin on the A_1-Cells of the Pancreatic Islets in Guinea-Pigs[1]

In all mammals the pancreatic islets are composed of 3 types of granulated cells, the A_1-cells, the A_2-cells and the B-cells. While it is generally agreed that the B-cells produce insulin[2] and the A_2-cells glucagon[3], the function of the A_1-cells is still unknown. It has been suggested, however, that the A_1-cells are connected with the gastrin production[4,5]. Recently LOMSKÝ, LANGR and VORTEL[6] have demonstrated, using immunochemical technique, that in man and some other species the A_1-cells show specific affinity for gastrin antibodies. In an earlier study, there was no evidence that gastrin injections had any effect on the A_1-cells in guinea-pigs[7]. These observations are here reevaluated, since synthetic gastrin is now available, as well as its terminal pentapeptide, which has been used in the present study.

Methods. 10 male guinea-pigs were injected s.c. with pentagastrin[1] every 3rd h for 5 days with a dosage of 4 µg each time for the first 4 days and then 6.5 µg. The substance was dissolved in saline. The animals were killed 1–2 h after the last injection by a blow on the head, followed by bleeding. Pieces of pancreas were immediately immersed in Zenker-formol, dehydrated and embedded in paraffin. The 7 µm thick sections of pancreas were silver-impregnated by a modified Davenport method[8]. The islets of Langerhans were photographed and the sections susequently restained with Gomori's chrome-hematoxylin ponceau fuchsin[9]. In the pancreas from each animal, the areas of the largest optical cross-section of 24 A_1, 24 A_2 and 24 B-cells were calculated after measuring the largest nuclear diameter, and that at right angles to the latter, by means of an ocular screw micrometer at a magnification of $\times 1875$ (cf. HELLMAN and HELLERSTRÖM[10]). The nuclei were collected from at least 5 islets in each animal. Only nuclei with their centres within the sections were measured.

The adrenal glands were weighed after 24 h of immersion in 10% formol. The blood glucose levels were determined by the glucose oxidase method[11].

Results. Total body weight showed no meaningful differences between the pentagastrin-treated guinea-pigs and the control animals (Table I). The mean value for the adrenal weights of the treated animals was significantly higher ($P < 0.05$) than the corresponding value for the controls (Table I). As can be seen in the same Table, the blood sugar values were about the same in the 2 groups.

Table I. Body weight, before the first and after the last injection, adrenal weight and serum glucose level

	Body weight (g)		Adrenal weight	Blood glucose
	Before	Afterwards	(mg)	(mg/100 ml)
Pentagastrin treated guinea-pigs	232 ± 8	263 ± 9	40 ± 1	89 ± 4
Controls	221 ± 6	243 ± 8	35 ± 1.5	86 ± 10
	$t = 1.09$	$t = 1.66$	$t = 2.70$	$t = 0.27$
	df = 18	df = 18	df = 18	df = 18
	$P > 0.05$	$P > 0.05$	$P < 0.05$	$P > 0.05$

Mean value $\pm$ S.E.M.

Table II. Nuclear size (arbitrary units) of the islet cells in the 2 groups of guinea-pigs

	A_1-cells	A_2-cells	B-cells
Pentagastrin treated guinea-pigs	0.48 ± 0.007	0.60 ± 0.010	0.52 ± 0.009
Controls	0.51 ± 0.009	0.62 ± 0.010	0.53 ± 0.009
	$t = 2.46$	$t = 1.10$	$t = 0.30$
	df = 18	df = 18	df = 18
	$P < 0.05$	$P > 0.05$	$P > 0.05$

Mean values $\pm$ S.E.M.

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