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PLASMA ADRENALINE, NORADRENALINE AND DOPAMINE IN MAN AND DIFFERENT ANIMAL SPECIES

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SUMMARY

1. The catecholamines adrenaline (A), noradrenaline (NA) and dopamine (DA) were determined in plasma samples of man and various animal species using a highly sensitive radioenzymatic method.

2. Basal values were determined under conditions producing virtually no physical or psychic stress in blood obtained through acutely inserted venous catheters in human volunteers, rabbits and cows, through chronic indwelling catheters in cats and rats, and by cubital venipuncture in trained dogs.

3. Basal values (pg/ml.) for A, NA, and DA were respectively 64, 203 and 98 in man, 73, 609 and 276 in cats, 166, 392 and 216 in rabbits, 56, 152 and 91 in cows, 204, 376 and 173 in dogs, and 175, 509, and 84 in SPF rats. The NA concentrations were always higher than those of A and DA.

4. Gentle handling of rats for 30 sec greatly increased the levels of all catecholamines, especially of A. Even more marked rises were observed during and up to 5 min after restraint stress.

5. Blood from the trunk of decapitated rats contained about 20 times more A and 3–4 times more DA and NA than venous blood from catheters in the absence of handling.

6. Basal values of plasma catecholamines in small animals can only be obtained through indwelling catheters and in the absence of handling. Most of the previously reported values are too high and are experimental artifacts.

INTRODUCTION

The difficulties met when estimating the minute amounts of catecholamines present in the blood of man and various animal species are reflected by the divergent data found in the literature (Callingham, 1975). Great individual variations and factors concerned with techniques crucially affect the reliable measurement of the plasma catecholamine concentrations. Assay methods growing a higher specificity and an increased sensitivity, together with more appropriate ways of blood collection, must therefore be sought, in order to obtain accurate and comparable catecholamine determinations, as stressed by Callingham & Barrand (1976). In humans, improved

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assay procedures together with carefully standardized methods of blood collection have led to more consistent data in catecholamine studies (Callingham & Barrand, 1976). In contrast, the situation is still unsatisfactory in animal studies. This applies particularly to small animals, where the difficulty of defining a condition presenting no stress is associated with the need to collect amounts of blood that are large in proportion to the size of the animal (Callingham & Barrand, 1976).

Recently a highly specific and sensitive radiometric procedure was developed which allows the simultaneous determination of adrenaline (A), noradrenaline (NA) and dopamine (DA) in biological samples (Da Prada & Zürcher, 1976). Because of the high sensitivity of this method (1 pg for A and NA; 5 pg for DA), very small amounts of blood (0·4 ml.) are sufficient for the simultaneous estimation of the three catecholamines in plasma. This fact, associated with the use of techniques of blood drawing presenting no stress to the animal, provided a first step towards the assessment of the basal catecholamine levels in animal plasma, which is a prerequisite for any interpretation of their modifications following physiological and pharmacological manipulations. We here present basal plasma catecholamine values in man and in some animal species and the differences in plasma catecholamine concentrations in rats after collection by several procedures.

METHODS

Subjects

A total of eleven healthy male human volunteers aged 18–25, five cats, four burgundian rabbits, six german shepherd dogs, four cows, fifty male rats of 200–300 g (thirty-one of Wistar Füllinsdorf (SPF), six of Sprague–Dawley, six of Fawn-Hooded, seven spontaneously hypertensive rats Wistar-Kyoto strain, SHR) were the subjects of the present study. Blood drawings from all animal species tested were carried out at approximately 10 a.m. in order to avoid possible variations associated with the diurnal rhythms. Blood was collected via acutely or chronically implanted catheters in all species examined except for dogs.

Blood sampling

Human blood samples were obtained 30 min following implantation of catheters in the cubital vein and resting of the subject in the supine position. In the *cats*, a chronic catheter was implanted in the jugular vein under ether anaesthesia. In the *rabbits*, a polyethylene catheter was inserted in the marginal vein of the ear under local anaesthesia and blood samples were drawn when the frequency of the respiration monitored was normal in animals isolated and quietly resting in a sound-insulated cage. The *dogs* had long been trained to daily blood drawing by cubital venipuncture and presented little, if any signs of stress at the moment of blood collection. *Cows* had catheters implanted in the jugular vein. 30 min later blood samples were obtained from the animals standing completely undisturbed in their usual environment. The *rats* were housed in individual cages for a period of one week before use at a room temperature of ± 22 °C and were allowed access to food and water *ad lib*. A chronic silastic catheter was inserted in the jugular vein under light ether anaesthesia, according to the technique described by Brown & Hedge (1972). In some experiments a catheter was implanted in the carotid artery, under otherwise identical conditions. Since preliminary data indicated that plasma catecholamine concentrations did not significantly differ in the same animal one to seven days after catheter implantation, blood samples were collected one day after surgery. The blood samples were collected through a polyethylene cannula, 15 cm in length, which was connected to the chronic jugular or carotid catheter 30 min before the blood drawing. Each time the blood volume drawn (0·4 ml.) was replaced with saline. During blood collection the rats were allowed to move freely in their individual cages and appeared to be completely unaware of the procedure. Values obtained under these conditions were considered the basal ones. In one series of experiments jugular blood samples were obtained from four chronically cannulated SPF rats (1) while they were

being gently handled for at least 30 sec, and 30 min after replacement in their basal individual cages conditions, (2) after 5 min of exposure to the restraint stress by immobilizing the animal's trunk in an aluminium mesh as described by Bonfils (1964). Plasma catecholamine concentrations were also measured following a presumptive situation of very strong stress represented by scissor decapitation and they were compared with the basal concentrations.

Catecholamine assay

The blood samples (0·4 ml.) were collected into a plastic syringe containing heparin (Liquemin, Roche; 10 NIH units/ml.). Immediately after collection the samples were transferred to 0·5 ml. polypropylene tubes, cooled in a water bath (~ 6 °C) and centrifuged for 2 min in a Beckman Microfuge at room temperature. The plasma samples were then collected, frozen on dry ice and stored at − 80 °C until catecholamine estimation was carried out. A, NA and DA were assayed simultaneously in duplicate using 100 µl. plasma by the radioenzymatic method of Da Prada & Zürcher, (1976). Basal DA values were at least twice, A and NA 4–5 times the blank value. Internal standards were usually run in two samples per day. (The inter-assay variability was ± 18 % for A, ± 3, 7 % for NA and ± 2, 6 % for DA). In brief, the catecholamines were converted to their *O*-methylated labelled derivatives by catechol-*O*-methyl-transferase in the presence of tritiated-S-adenosyl-methionine (Radiochemical Centre, Amersham). The radioactive methylated derivatives were then extracted and separated by thin-layer chromatography. [^{3}H]-methoxytyramine was scraped off and assayed directly for its radioactivity. The tritiated metanephrine and normetanephrine were first converted to vanillin by periodate cleavage, and thereafter extracted into toluene and their radioactivity measured by an ISOCAP/300 liquid scintillation spectrometer (Nuclear Chicago).

Statistical evaluation of differences between experimental groups was performed using the Kolmogorov-Smirnov test (two-tailed) (Campbell, 1971).

RESULTS

The concentrations of the plasma catecholamines determined in the venous blood of humans and various animal species under basal conditions are reported in Table 1. It can be seen that with the experimental procedures used to minimize the stress connected with blood collection, the plasma levels of A, NA and DA were very low (below 1 ng/ml. plasma) in all species examined, in contrast to the higher values reported in the literature from unanaesthetized animals (reviewed by Callingham, 1975). The lowest mean values of plasma A and NA were found in the resting supine human subjects (64 and 203 pg/ml. respectively) and in cows (56 and 152 pg/ml.). The highest concentration of A was detected in the dog, the highest value for NA in the cat. DA was present in the plasma of all species examined, its mean values ranging from 84 pg/ml. in the SPF rats to 276 pg/ml. in the cat.

In all species examined, mean values of NA constantly exceeded those of A and DA (Table 1). Moreover, in spite of a fairly wide range of individual variations, the plasma of each animal invariably contained more NA than A or DA.

In four different strains of rats low plasma catecholamine concentrations were found when the blood was collected from a chronically implanted jugular catheter in the unrestrained situation (Table 2). Under these experimental conditions, the concentrations of NA were two or more times those of A and DA. Due to high individual variations, no statistically significant difference was found in the catecholamine content of different strains of rats. However, low values of NA were found in SHR rats as compared with the SPF, Fawn-Hooded and Sprague–Dawley rats.

Extremely high catecholamine concentrations were found in the first blood emerging from the trunk of unanaesthetized rats decapitated with scissors. The A values were

TABLE 1. Plasma concentrations of adrenaline (A), noradrenaline (NA) and dopamine (DA) in man and some animal species

	Method of blood collection	Number of subjects	A	NA	DA
Man	a	11	64 ± 5 (47–71)	203 ± 10 (167–220)	98 ± 20 (41–131)
Cat	b	5	73 ± 17 (30–113)	609 ± 119 (282–912)	276 ± 7) (276–297)
Rabbit	c	4	166 ± 39 (120–283)	392 ± 33 (300–504)	216 ± 23 (164–259)
Dog	d	6	204 ± 12 (170–239)	376 ± 37 (278–524)	173 ± 61 (20–437)
Cow	b	4	56 ± 11 (26–70)	152 ± 12 (132–180)	91 ± 35 (26–183)
Rat (SPF)	b	18	175 ± 30 (25–391)	509 ± 46 (277–783)	84 ± 9 (36–151)

Results are mean values ± S.E. of means and are expressed in pg/ml. of plasma, their ranges being indicated in parentheses.

Method of blood collection: a, 30 min after catheter implantation in the cubital vein in the supine position; b, from a chronic catheter in the jugular vein; c, from a catheter implanted in the marginal vein of the ear; d, by venipuncture in the cubital vein.

TABLE 2. Plasma concentrations of adrenaline (A), noradrenaline (NA), and dopamine (DA) in the blood obtained from freely moving rats of different strains through chronic jugular catheters or by decapitation

Rat strain	Number rats	A	NA	DA
Freely moving				
Sprague–Dawley	6	120 ± 25 (42–185)	· 461 ± 60 (219–648)	188 ± 25 (109–291)
Fawn-Hooded	6	256 ± 72 (162–471)	451 ± 73 (348–660)	122 ± 46 (48–225)
SHR Wistar	7	110 ± 16 (66–153)	318 ± 67 (130–525)	85 ± 14 (57–135)
SPF Wistar origin	18	175 ± 31 (23–415)	509 ± 46 (230–783)	84 ± 9 (36–151)
Decapitated				
SPF Wistar origin	6	3014 ± 308* (2051–4242)	2012 ± 181* (1374–2757)	234 ± 15* (170–308)

Results are mean values ± S.E. of means and are expressed as pg/ml. plasma. Range in parentheses. Significance SPF decapitated vs. catheterized $P < 0{\cdot}001$*.

about twenty times higher than in the freely moving rats, whereas those of NA and of DA were, respectively, about four and three times higher (Table 2).

As shown in Table 3, the catecholamine concentrations measured in the arterial blood obtained from a catheter inserted in the carotid artery did not significantly differ from those found in the jugular blood in SPF freely moving rats.

The basal values of plasma A obtained from the venous blood of freely moving

TABLE 3. Plasma concentrations of adrenaline (A), noradrenaline (NA) and dopamine (DA) in blood obtained from chronic jugular or carotid catheters in freely moving SPF rats

	Number of rats	A	NA	DA
Jugular vein	8	175 ± 44 (41–391)	502 ± 68 (308–783)	91 ± 12 (36–142)
Carotid artery	8	374 ± 80 (113–858)	877 ± 146 (413–1403)	85 ± 14 (29–156)

The results represent mean values ± S.E. of means and are expressed as pg/ml. plasma. The range is given in parentheses. The differences between arterial and venous blood are not statistically significant ($P > 0.05$).

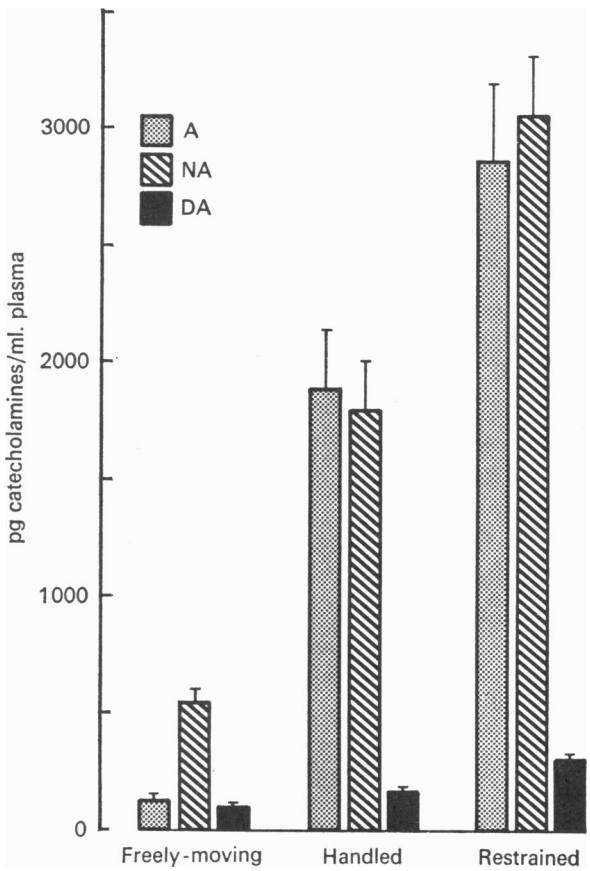

Fig. 1. Basal concentrations of adrenaline (A), noradrenaline (NA) and dopamine (DA) in the plasma of freely moving SPF rats with indwelling jugular catheters and their changes following handling and restraint stress. Values are the means ± S.E. of means for four rats. The basal values as well as the values after 30 sec of handling and of 5 min restraint stress were determined in blood samples collected at 30 min intervals through a chronic catheter implanted in the jugular vein. Differences in A, NA, and DA between freely moving, undisturbed rats and handled animals are statistically significant ($P < 0.01$).

SPF rats were compared with those found in the blood collected while the rat was either handled or restraint-stressed (Fig. 1). Gentle handling of the rat for as short as 30 sec caused the plasma A concentration to rise to about 1000 % of the basal values, whereas the percentage increases of NA and DA were about 300 % and 100 % respectively. Restraint stress further increased significantly all three catecholamine levels, with approximate increases of 2000 % for A, 600 % for NA, and 300 % for DA. (Fig. 1).

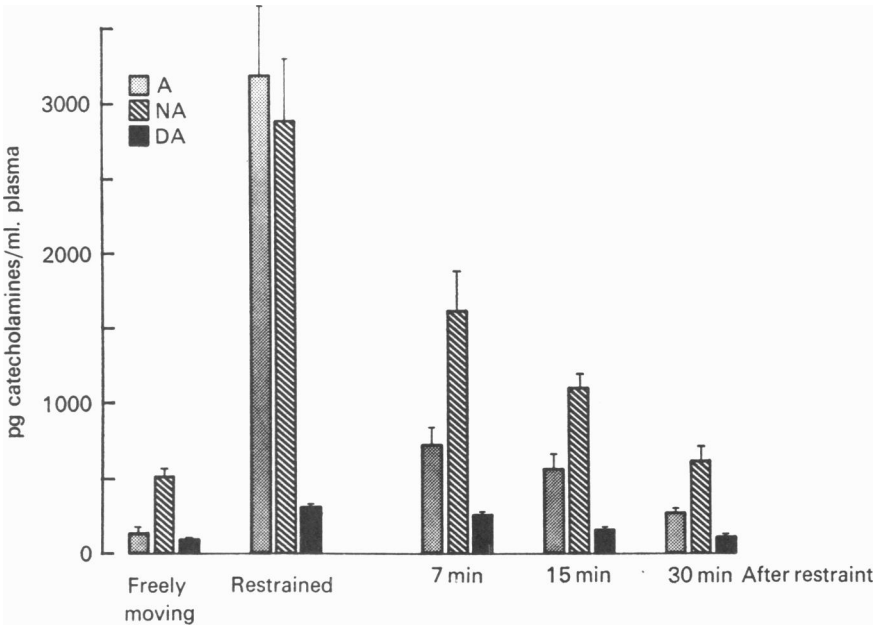

Fig. 2. Plasma adrenaline (A), noradrenaline (NA) and dopamine (DA) concentrations in the jugular blood of SPF rats prior to, during, and after restraint stress of 5 min duration. Values are the means ± S.E. of means for three rats. The blood samples were drawn through chronically implanted jugular catheters while the animals were undisturbed in their cages immediately before the restraint stress procedure, at the end of a 5 min restraint, and 7, 15 and 30 min after placing the rats back in their cages. Values of A, NA, and DA in freely moving, undisturbed rats are significantly different from those in animals subjected to restraint stress ($P < 0{\cdot}01$).

Fig. 2 shows the increases of plasma A, NA, and DA following 5 min of restraint stress as compared with the basal values in the free-moving situation, as well as the time course of the return to basal levels after placing the rats back in their individual cages. The plasma catecholamine concentrations were also measured in the blood emerging from the trunk following decapitation of three SPF rats with jugular catheters subjected to restraint stress for the 5 min preceding decapitation. The extraordinarily high concentrations of catecholamines found were 24,482 ± 5,193, 15,925 ± 4,423 and 1,427 ± 315 pg/ml. plasma, for A, NA and DA respectively.

DISCUSSION

In the present study of plasma catecholamines in several animal species, which made use of the newly developed and extremely sensitive method for catecholamine

assay of Da Prada & Zürcher (1976) and of techniques of blood drawing which minimize or prevent stress, levels of A, NA and DA in the unanaesthetized animal were found to be lower than those reported in the literature (see Callingham, 1975). Under our conditions the mean values of NA were consistently higher than those of either A or DA in all species examined. Within each species an interindividual variability of catecholamine levels was observed which is at least in part attributable to various levels of sympatho-adrenal activity and/or of reactivity to environmental stimuli at a given moment. This view is strengthened by the fact that in freely moving rats with jugular catheters the levels of the amines did not significantly vary from day to day. In spite of the individual variability observed, the resting concentrations of NA in each animal tested invariably exceeded those of A and of DA, the DA level being usually higher than that of A.

The plasma content of A and NA measured in healthy humans under resting conditions is comparable with that recently reported in the literature (Callingham, 1975; Weise & Kopin, 1976). Only a few estimates of DA plasma levels in man have been published, and these ranged from 230 pg/ml. (Christensen, 1973) to 403 pg/ml. (Peuler & Johnson, 1975). In the present study lower concentrations of DA were found (range 41–131 pg). This difference is possibly attributable to the lower specificity of the methods employed as compared to ours. Weise & Kopin (1976) have recently reported plasma levels of DA from humans in the range of 0–79 pg/ml., but these data were not considered by the authors to be reliable for technical reasons.

The plasma catecholamine values in various animal species obtained under our experimental conditions of minimal or absent stress are in general far lower than the values found in the literature, which, moreover, show a larger variability. It seems that this discrepancy is largely due to the fact that most, if not all previous investigators were sampling blood under stressful conditions. In the case of plasma catecholamines measured in anaesthetized animals, both higher and lower values were reported than found in the present study. The reason for these discrepancies might be the different effects of anaesthetics on the activity of the sympathoadrenal system. For instance, Street & Roberts (1969) determined A and NA plasma values of 1·66 and 1·15 ng/ml. respectively in chloralose-anaesthetized cats, as compared with the plasma values of 73 pg/ml. for A and 609 pg/ml. for NA found in the unanaesthetized cat in the present study. Eleftheriou (1974) found about 9·0 ng/ml. of NA in the plasma of decapitated rabbits, compared with the present result of 392 pg/ml. Anton & Sayre (1962) reported concentrations of 410 pg/ml. for A and 185 pg/ml. of NA in dog plasma while the present values were 204 pg/ml. and 376 pg/ml. respectively, obtained in plasma of dogs trained to venipuncture and therefore in a situation of low stress. Lower values than these for both A and NA (100 pg/ml. and 180 pg/ml. respectively) were found in the dog when the blood was drawn under thiopentone anaesthesia (Millar, Keener & Benfey, 1959). Martin & Harrison (1968) found 76 pg/ml. and 1 ng/ml. of A and NA respectively in the calf under barbiturate anaesthesia, whereas in the present experiments 56 pg/ml. of A and 152 pg/ml. of NA were found in the conscious cow.

More variable and considerably higher values, in some instances even exceeding 10 ng total catecholamines/1 ml., with concentrations of NA up to 1 ng, were reported by a number of authors in the plasma of rats of different strains, such as the Sprague–

Dawley (Roizen, Weise, Moss & Kopin, 1975; Grobecker, Roizen & Kopin, 1977), Wistar (Dargie, Franklin & Reid, 1976) and Long-Evan rats (Ben-Jonathan & Porter, 1976) which were killed by decapitation. Lower catecholamine values were reported when the blood was obtained under the effect of various anaesthetics (Roizen, Moss, Henry & Kopin, 1974; Green & Miller, 1966).

The extremely high concentrations of plasma catecholamines found by Roizen *et al.* (1975) in rats, either when drawing blood from arterial catheters or collecting it from the bleeding trunk after decapitation, can only be accounted for by the unequivocal stressful situation associated with both ways of blood collection. In fact, more recently the same group (Chiueh, Popper & Kopin, 1976) has reported much lower values of plasma A and NA in chronically catheterized rats. That in rats simple handling may by itself cause a significant increase of plasma A, NA and DA concentrations is demonstrated by the present experiments, which also show that a condition of more severe stress causes a further and longer lasting enhancement of the concentrations of all three catecholamines. Most previous reports by various investigators, including those who compared plasma catecholamine concentrations in hypertensive and normotensive rats in blood collected by decapitation, have to be considered against this background. The divergent findings of Grobecker, Roizen, Weise, Saavedra & Kopin (1975) (higher NA concentration in plasma of SHR versus normotensive Wistar Kyoto), and of Nagaoka & Lovenberg (1976) (no appreciable differences between hypertensive and control rats), could be due to some variable in the handling and decapitation procedure. When collecting blood from indwelling catheters, we found the resting concentrations of NA in freely moving, spontaneously hypertensive rats of the Kyoto strain (SHR) not significantly different from those in normotensive rats of other Wistar or of Sprague–Dawley strains.

In future investigations of experimentally induced modification of plasma catecholamines or in comparative studies of different groups of animals, the importance of careful consideration of potential sources of undesired stress cannot be overemphasized. Catheterization, which is easily performed in the rat, may permit the collection of blood from unrestrained animals and, thus the detection of plasma catecholamine concentrations associated with different behavioural situations. Other authors have recently reached similar conclusions for the resting level of plasma NA (Depocas & Behrens, 1977).

Combining the very sensitive method of Da Prada & Zürcher (1976) with chronic catheterization has the advantage that no more than 50 μl. plasma per sample is needed (the required minimum being 25 μl.) and that the time course of induced changes of the blood catecholamines can be followed. The time course of the blood catecholamine changes during general anaesthesia produced by various agents is at present being studied.

Under the nearly physiological conditions used in this study, DA was invariably found in the plasma of all species examined. Thus DA is very likely to be a normal constituent of mammalian plasma. In different species and various strains of rats, basal plasma DA concentrations were low and variable, with the highest range in the dog (20–437 pg/ml.). Similar plasma DA concentrations (100–400 pg/ml.) were reported by Kelly, Sharman & Tegerdine (1970) in sheep and goats, whereas exceedingly high plasma values in the chloralose-anaesthetized cat were found by Street &

Roberts (1969). The origin of plasma DA is not yet known. Secretion of DA from isolated perfused adrenals of sheep has been demonstrated by Lishajko (1970) and a resting secretion of DA into the adrenal venous effluent was reported by Hempel & Männl (1967) in the cat anaesthetized with ether. The above data show that both handling and restraint stresses in rats cause significant increases in plasma DA concentrations which, however, are less than those of A and NA. Further, preliminary experiments have shown reduced but still detectable amounts of DA and no traces of A in adrenalectomized rats as compared with values in sham-operated controls; the blood samples were obtained by decapitation 24 and 72 hr following adrenalectomy (Picotti, G. B. & Da Prada, M., unpublished). This suggests that in the rat the plasma DA is only partially of adrenal origin. The relative contribution of other peripheral tissues to plasma DA is also under investigation in this laboratory.

Some of these data were part of a study submitted by Dr H. U. Bühler in an M.D. thesis at the University of Basel, Switzerland or have been presented at the Spring Meeting of the Union of Swiss Societies of Experimental Biology (Picotti, Bühler, Da Prada, 1977) and at the XXVIIth Int. Congr. Physiol. Sci. (Da Prada, Picotti, Bühler, Haefely, 1977).

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