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THE EFFECT OF DIFFERENT DOSES OF DIMETHYLBIGUANIDE (DMB) ON LIVER BLOOD FLOW, BLOOD GLUCOSE AND PLASMA IMMUNOREACTIVE INSULIN IN ANAESTHETIZED RATS

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Abstract—The effect of different doses of dimethylbiguanide (DMB) administered by the i.v. and i.d. route on liver blood flow, blood glucose and plasma immunoreactive insulin was investigated in anaesthetized rats. Liver blood flow was measured by thermocouples implanted into the liver utilising Grayson's principle of "internal calorimetry". In addition blood glucose and immunoreactive insulin in the plasma was measured at different time intervals following the different doses of DMB. Intraarterial BP was monitored during the whole experiment. Following 100 mg/kg DMB i.v. a significant increase in liver blood flow, blood glucose, plasma immunoreactive insulin and BP was observed. 50 mg/kg DMB i.v. did not affect any of the parameters measured, while 150 mg/kg DMB was already a toxic dose. After i.d. administration no changes were seen following a dose of 175 mg/kg DMB and 1500 mg/kg DMB was already toxic. 500 mg/kg DMB, however, showed a similar significant increase in liver blood flow, blood glucose and plasma immunoreactive insulin as 100 mg/kg DMB i.v. These effects observed could be suppressed by a simultaneous administration of 1 mg/kg propranolol i.v. In addition a decrease in liver blood flow occurred which is explained by a direct effect of the β-adrenergic blocking agent propranolol on the liver vasculature. Therefore a selective β-adrenergic effect of the biguanides seems a likely explanation for the increased liver blood flow following DMB administration.

Administering 1.6 g/kg glucose i.d. an increase in liver blood flow, blood glucose and plasma immunoreactive insulin was observed. This influence on blood glucose and plasma immunoreactive insulin was inhibited by simultaneous administration of 1.6 g/kg glucose and 500 mg/kg DMB, while liver blood flow showed the same increase when glucose alone was given. After changing the experimental design in giving the 500 mg/kg DMB i.d. and 30 min later 1.6 g/kg glucose a much higher increase in liver blood flow occurred, which might reflect an additive effect.

The blood glucose lowering effect of biguanides compatible with all observations in animals and man, like the inhibition of the gastric motility, the decreased glucose absorption from the small intestine and the inhibition of hepatic gluconeogenesis, is not completely understood [1–6]. In addition, different and partly opposite effects on insulin secretion were observed, depending on the dosage administered and on the species in which the experiments were performed [6–9]. However, a recently developed molecular theory based on experimental evidence, relates the side of action of biguanides to biological membranes in a rather unspecific manner [10]. From this molecular mechanism of interaction with membranes a large multiplicity of possible consequences is selfevident and many different findings, including species differences may well be explained on the basis of the membrane modifying capability of the biguanides. Therapeutic doses of biguanides in man caused no increase in insulin secretion, while following phenyl- and dimethyl-biguanide an increase in insulin levels without changes in blood sugar was seen in rats and dogs [11, 12]. This increase in insulin could be blocked by β-adreno-receptor blocking agents and therefore it was assumed, that biguanides do not directly stimulate the pancreatic β-cells, but affect the β-adrenergic nervous system. This assumption might be supported by the fact of an increased liver blood flow and mean arterial pressure found in dogs, following long term treatment by dimethyl-biguanide in a dosage used therapeutically in man [13]. Therefore, in the present study in acute experiments the effect of different doses of DMB on blood glucose, insulin secretion and liver blood flow was investigated in rats. In addition, the influence of glucose alone and the simultaneous administration of glucose and DMB on the parameters mentioned above was investigated.

MATERIALS AND METHODS

Forty eight male Wistar rats which weighed 200–300 g and had fasted overnight for 16 hr were used in this study. Following Inactin anaesthesia (60 mg/kg i.p.) the right jugular vein was used for intravenous (i.v.) injections and for obtaining blood samples at different time intervals. The carotid artery was used for monitoring the blood pressure (BP) by

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a statham transducer element (type P 23AC). Body temperature was recorded rectally throughout the experiment by an electronic thermometer (Sekunden-Thermometer, Quarz A. G., Zürich). Liver blood flow was measured according to the principle of "internal calorimetry" utilising an improved apparatus previously described [14, 15]. This method uses heated thermocouples, whereby current is supplied to the heaters of the thermocouple in cyclic fashion as follows: 0.2 A^2 for 34 sec; 0.6 A^2 for 17 sec; 0.315 A^2 for 24 sec; no current for 17 sec. This cycle lasts for about 1.4 min and is continuously repeated. The difference between the two equilibria 0.315 A^2 and 0.2 A^2 is recorded on a potentiometric recorder and gives the temperature increment (θ) produced by a current of 0.115 A^2 (I^2). Changes in liver blood flow decrease or increase the distance between both equilibria. From these recordings the thermal conductivity (K) can be determined according to the relation.

$$I^2 = F \cdot \theta \cdot K$$

whereby F is a known instrumental constant and θ can be measured from the record. Grayson and Johnson [16] have found in rats that the conductivity is a linear function of liver blood flow in the physiological range and therefore an increase in conductivity is comparable to an increase in liver blood flow and vice versa. The baseline conductivity found in each individual rat shows variations which can be explained by the position of the thermocouple, to the liver blood vessels. A thermocouple located near a bigger vessel gives a higher baseline conductivity than in a low flow area [16]. Therefore the differences were statistically analysed in each individual rat. The baselines of the different rats were set at 100 per cent and the mean increases expressed as mean percentages. Further details, the evaluation of the method and calculations including statistical analysis are given elsewhere [17].

The heaters of the thermocouples were implanted in the middle of the right lobe of the liver following a ventral midline incision to expose the upper part of the abdominal cavity. In addition a small polythylene catheter was inserted in the lower part of the duodenum for the instillation of the different doses of DMB and glucose used in this study. After the operation the abdominal wall was closed by means of a few sutures. There were no experimental procedures done for 1 hr so that steady state conditions could be obtained. After 1 hr a blood sample of 50 μl for the determination of blood glucose and plasma immunoreactive insulin was taken. Liver blood flow measurement was started for the following 30 min.

These values were taken as controls and all other values were compared with these baseline values. Following this control period DMB as an aqueous solution was given, i.v. or by intraduodenal (i.d.) installation in doses as listed below:

50 mg/kg DMB i.v.
100 mg/kg DMB i.v.
150 mg/kg DMB i.v.
175 mg/kg DMB i.d.
500 mg/kg DMB i.d.
1500 mg/kg DMB i.d.
500 mg/kg DMB i.d. + 1 mg/kg propranolol i.v.

In addition glucose or the following combinations were administered:

1.6 g/kg glucose i.d.
500 mg/kg DMB + 1.6 g/kg glucose i.d. as simultaneous administration.
500 mg/kg DMB i.d., followed 30 min later by 1.6 g/kg glucose.

All doses were dissolved in 1–2 ml of distilled water. Following the administration of the different doses of DMB or the different combinations with glucose and propranolol, 50 μ blood samples were taken after 30, 60, 90 and 120 min. This determined the level of glucose and plasma immunoreactive insulin. Blood glucose levels were measured by means of the hexokinase method using reagents from Boehringer Mannheim, Germany [18]. Plasma insulin was estimated by a widely used radioimmunological method, with porcine insuline as a tracer and an antiserum raised against porcine insulin [19]. The standard was purified rat insulin (batch no. R 170, kindly provided by Dr. L. G. Heding, Novo Research Institute, Kopenhagen) with a biological activity of 21.4 U/mg and the results are expressed as pg/ml of that standard. The interassay variation of the radioimmunoassay was about 12 per cent using every day the same samples as a quality control.

Statistical analysis was done using a one-way analysis of variance if the variances of the means were found to be homogenously distributed as seen by the Bartlett–Test. Some of the glucose mean values at certain time intervals and all of insulin mean values, showed an inhomogenous distribution of variance. Therefore a Kruskal–Wallis–H–Test as a non-parametric test was used and all insulin values are given as the median and its corrected S.D. [20]. The values of thermal conductivity measured in cycles of about 1.4 min duration in the 30 min periods were calculated by a one-way analysis of variance to obtain the difference of each period in each individual rat. The changes in thermal conductivity are given as a mean increase in percentage for reasons described above.

RESULTS

The results of the changes and the time course observed in liver blood flow, blood glucose and plasma immunoreactive insulin following the different doses of DMB and combinations with glucose are given in Tables 1, 2 and 3. The blood glucose and insulin values measured in the control period in the different groups of rats were tested by a Kruskal–Wallis–H–Test and showed no significant differences between the different groups. A mean value of 125.2 mg/100 ml ± 40.5 S.D. glucose was found, while the immunoreactive insulin comparable to these glucose values was 336.6 pg/ml ± 167.2 S.D. or 7.41 μU/ml ± 3.68 S.D. The mean arterial BP of all control periods was 104 ± 10.4 mmHg. Significant changes in BP were seen only following 100 mg/kg DMB i.v. going up to a level of 121 mmHg 60 min after drug administration. After 1500 mg/kg a significant drop in BP down to 63 mmHg was seen. In all other groups of rats no significant changes in BP were observed.

Table 1. The influence of different doses of dimethylbiguanide on thermal conductivity as a measurement of *liver blood flow* following i.v. or i.d. administration

Doses of dimethylbiguanide administered	Changes in % of baseline conductivity 30 min	60 min	90 min	120 min
100 mg/Kg DMB i.v. $n = 6$	+17.6	+19.6	+12.2	+8.1
175 mg/Kg DMB i.d. $n = 6$	0	0	0	0
500 mg/Kg DMB i.d. $n = 6$	+10.1	+22.8	+24.9	+15.4
1500 mg/Kg DMB i.d. $n = 6$	−36.5	−27.0	—	—
500 mg/Kg DMB i.d. + 1 mg/Kg propranolol i.v. $n = 6$	−5.4	−4.1	−18.4	−12.0
1.6 g/Kg glucose i.d. $n = 6$	+16.3	+33.3	+52.2	+50.1
1.6 g/Kg glucose + 500 mg/Kg DMB i.d. simultaneously $n = 6$	0	+37.1	+58.6	+59.4
500 mg/Kg DMB i.d. + 30 min later 1.6 g/Kg glucose i.d. $n = 6$	0	+71.7	+85.4	+83.0

All increments given in this Table are significant ($P < 0.01$–0.001).

Following an i.v. injection of 50 mg/kg DMB no changes in any of the parameters measured could be seen. Following 150 mg/kg DMB i.v. the animals died after 15–20 min showing the signs of a severe dyspnoa followed by a central apnoa. In contrast following the administration of 100 mg/kg DMB i.v. an increase in thermal conductivity of 18 per cent over baseline values occurred already 30 min after drug administration. This effect was further enhanced after 60 min up to about 20 per cent and could be still observed at the end of the experiment. This increase in thermal conductivity was seen in all rats investigated and was highly significant ($P < 0.01$–0.001). Combined with this increase in thermal conductivity a significant increase in blood glucose was seen after 30, 60 and 90 min and with a return to baseline values at 120 min. In addition a simultaneous significant increase in plasma insulin values at 60 and 90 min occurred. The mean arterial pressure rose from 107 to 121 mmHg and was significantly increased after 60 min.

Following i.d. administration of 175 mg/kg DMB no changes in the parameters measured occurred. In contrast after 500 mg/kg DMB given by the i.d. route a significant increase in thermal conductivity in all rats investigated was observed ($P < 0.01$–0.001). This increase reached its maximal effect of 25 per cent above baseline value after 90 min and was accompanied by a significant increase in blood glucose after 60 and 90 min. Increases in plasma immunoreactive insulin were delayed and showed significant values at 90 and 120 min. Also the BP showed an increase up to 120 mmHg, but these levels were not significant at the 5 per cent level. In contrast, following an i.d.

Table 2. The influence of different doses of dimethylbiguanide on *blood glucose levels* following i.v. or i.d. administration

Doses of dimethylbiguanide administered	Mean ± S.D. (mg/100 ml) control	30 min	60 min	90 min	120 min
100 mg/Kg DMB i.v. $n = 6$	137 ± 41	169 ± 54*	175 ± 42†	176 ± 52‡	152 ± 51
175 mg/Kg DMB i.d. $n = 6$	136 ± 50	146 ± 47	154 ± 43	158 ± 41	140 ± 33
500 mg/Kg DMB i.d. $n = 6$	151 ± 44	188 ± 56	221 ± 39‡	197 ± 41*	178 ± 40
1500 mg/Kg DMB i.d. $n = 6$	107 ± 37	114 ± 46	93.5 ± 59	—	—
500 mg/Kg DMB i.d. + 1 mg/Kg propranolol i.v. $n = 6$	149 ± 42	171 ± 21	141 ± 32	102 ± 38	87 ± 36
1.6 g/Kg glucose i.d. $n = 6$	95 ± 28	129 ± 34*	150 ± 32‡	169 ± 69*	189 ± 40‡
1.6 g/Kg glucose + 500 mg/Kg DMB i.d. simultaneously $n = 6$	115 ± 43	159 ± 57*	177 ± 93	166 ± 50	151 ± 52
500 mg DMB i.d. + 30 min later 1.6 g glucose i.d. $n = 6$	144 ± 28	308 ± 87*	268 ± 86	236 ± 66	166 ± 60

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

Table 3. The influence of different doses of dimethylbiguanide on plasma immunoreactive insulin following i.v. or i.d. administration

Doses of dimethylbiguanide administered	Median ± S.D. (pg/ml) control	30 min	60 min	90 min	120 min
100 mg/Kg DMB i.v.	400	2180	2860	2390	1080
$n = 6$	± 163	± 1569	± 1119†	± 1427†	± 795
175 mg/Kg DMB i.d.	216	160	240	222	220
$n = 6$	± 47	± 55	± 95	± 52	± 47
500 mg/Kg DMB i.d.	180	436	530	1210	1200
$n = 6$	± 100	± 163	± 179	± 423*	± 520*
1500 mg/Kg DMB i.d.	344	3010	15000		
$n = 6$	± 117	± 1601*	± 3421*	—	—
500 mg/Kg DMB i.d. + 1 mg/Kg propranolol i.v.	396	360	264	188	255
$n = 6$	± 113	± 184	± 75	± 62	± 84
1,6 g/Kg glucose i.d.	284	270	424	1166	1064
$n = 6$	± 85	± 145	± 229	± 218*	± 239*
1.6 g/Kg glucose + 500 mg/Kg DMB i.d. simultaneously	337	1110	1020	822	730
$n = 6$	± 155	± 685	± 772	± 508	± 407
500 mg/Kg DMB i.d. + 30 min later 1.6 g glucose i.d.	480	980	5400†	1510	530
$n = 6$	± 221	± 403	± 1496	± 607	± 177

S.D.: for the median corrected S.D. of the arithmetic mean.
* $P < 0.05$.
† $P < 0.01$.

dose of 1500 mg/kg DMB the thermal conductivity dropped very rapidly to −36.5 per cent below baseline control values and remained there for 60 min. After that, all animals died, again with signs of severe dyspnoa followed finally by central apnoa. This decrease in thermal conductivity was parallel to a significant decrease in mean arterial BP from 105 to 63 mmHg and to a significant increase in plasma immunoreactive insulin to the highest levels observed in this study at the 30 and 60 min time interval.

Administering 500 mg/kg DMB by the i.d. route and giving simultaneously 1 mg/kg propranolol i.v. a slight decrease in thermal conductivity was observed already after 30 min and reached the lowest value of −18.4 per cent below baseline control after 90 min. Blood glucose and plasma immunoreactive insulin showed no changes at any of the time intervals investigated. Also the mean arterial pressure was not affected by this combination. Propranolol however abolished the effect of 500 mg/kg DMB observed after i.d. administration.

In the experiments administering an i.d. glucose load of 1.6 g/kg a significant increase in thermal conductivity was found already after 30 min. This increase reached its maximal effect up to 52 per cent over baseline readings after 90 min and could still be observed after 120 min. A similar time course was also seen in the blood glucose levels and a maximal value of 189 per cent was observed after 120 min. In contrast the plasma insulin levels showed a delayed rise and significantly increased values were reached after 90 and 120 min, even though the tendency to rise was seen earlier. BP changes did not occur. After administration of 1.6 g/kg glucose together with 500 mg/kg DMB simultaneously by the i.d. route no change in thermal conductivity was seen after 30 min. However, after 60–120 min an increase of about 59 per cent in thermal conductivity could be observed which was related only to a small significant rise in blood glucose at the 30 min time interval. Changes in plasma immunoreactive insulin were not significant because of the wide variation and the dissimilar time course of the values estimated in the individual rats. Changes in mean arterial BP were not observed in these experiments.

After changing the experimental design by giving 500 mg/kg DMB i.d. and 30 min later 1.6 g/kg glucose a similar time course occurred in the results when DMB and glucose were given simultaneously. However, the variations in blood glucose and plasma immunoreactive insulin were more extensive in the present experiments. Therefore, despite the very high blood glucose levels observed, only the 30 min values were significantly higher using a non-parametric distribution test for differences. In addition the plasma immunoreactive insulin levels showed the same variation and a significant increase at 60 min. In contrast, the thermal conductivity remained at first unchanged within the first 30 min period, but afterwards a very high rise in thermal conductivity up to 80 per cent occurred between 60 and 120 min. These changes were very constant in all rats investigated and highly significant at all time intervals ($P < 0.001$).

DISCUSSION

In the present study, the baseline control glucose and insulin levels measured showed a wide variation in the different groups investigated and the glucose values observed are in between the values found for conscious fasting and fed rats and should be regarded as non physiological, while the insulin values were in a similar range like in other studies in rat and man [7, 11, 21, 23]. These observations are probably due to the effects of anesthesia and operation in the abdominal cavity as similar values were reported in pentobarbitone anaesthetised and stunned rats, but the variations found in the different groups investigated were also found in other studies in conscious rats and man [22, 11, 23]. However, the higher base-

line values found in the present study remain constant throughout those experiments in which doses not effective on blood glucose and insulin levels were administered. Despite the wide variation in the different groups a Kruskal–Wallis–H–Test, a non parametric test, was used to assess statistic significance.

The changes in thermal conductivity as a semiquantitative measurement of liver blood flow, blood glucose, plasma immunoreactive insulin and mean arterial pressure observed were dependent on the route of administration and the different doses of DMB used. A dose of 175 mg/kg DMB given by a duodenal tube had no effect on blood glucose levels, as already found by Losert *et al.* [7]. In contrast following 500 mg/kg DMB i.d. and 100 mg/kg DMB i.v. a significant increase in blood glucose, immunoreactive insulin and liver blood flow was found. These changes were blocked by i.v. administration of propranolol, a β-adrenergic blocking agent. This observation provides some further evidence that the effect of DMB on blood glucose, insulin and liver blood flow is partly influenced by the β-adrenergic system. However, the increase in insulin might be additionally affected by the increases in blood glucose itself, observed after 100 mg/kg DMB i.v. and 500 mg/kg DMB i.d. On the other hand, the huge increase in insulin concentrations without affecting blood glucose following a dose of 1500 mg/kg DMB makes a blood glucose independent insulin stimulation much more likely.

The changes in insulin and blood glucose found in the present study are comparable to those seen in rats following phenformin administration [11]. These changes were thought to be due to adrenalin secretion of the adrenal glands as a complete disappearance of the phenformin induced increases in blood glucose and insulin was found after adrenalectomy. In addition, using the β-adrenergic blocking agent sotalol the phenformin induced increases in glucose and insulin were nearly completely reduced. Similar results were obtained in the present study after the simultaneous administration of the β-adrenergic blocking drug propranolol and DMB and these findings make an influence on the adrenergic system most likely. Therefore a stimulation of adrenalin secretion or a direct selective β-adrenergic effect on the hepatic vasculature both caused by biguanides could be a likely explanation for the increase in liver blood flow [24, 25]. β-Adrenergic receptors are present in the hepatic vasculature and were affected by the β-blocking drug propranolol causing an additional vasoconstriction of the portal vein and this resulted in a decrease of liver blood flow [25]. This decrease in liver blood was observed in the present study after administration of propranolol even in combination with DMB, which increased liver blood flow given alone.

Following glucose and the simultaneous administration of glucose and DMB an increase in liver blood flow as already found in dogs was observed [13, 26]. The increase in blood glucose following glucose administration were lowered by simultaneous DMB administration and this effect is explained by the known inhibition of glucose absorption in the small intestine [4, 5, 6]. According to this inhibition of glucose absorption blood glucose and insulin levels were lessened in the present experiments, but are comparable to results of an i.d. glucose tolerance test after DMB administration performed in man [27]. In the experiments in which 500 mg/kg DMB was given 30 min before i.d. glucose administration a higher increase in blood glucose was observed than after simultaneous administration of both substances. This might reflect an additive effect of DMB and glucose, as in addition a very high increase in liver blood flow of about 85 per cent compared to control levels was found.

In conclusion, the changes in the parameters measured in the present study were observed in anaesthetized and operated rats following high doses of DMB, but the alterations in glucose and insulin levels are comparable to those seen in conscious rats following phenformin administration [11]. Doses of DMB comparable to therapeutic doses used in man are not effective in rats [7] and therefore the results obtained in the present study do not necessarily apply to man. However, treating dogs being more sensitive to biguanides, by 15 mg/kg DMB, (a dose used therapeutically in man) similar changes in glucose, insulin and liver blood flow were found [13]. Therefore, despite different experimental procedures, animal species and biguanide substances and doses, the same tendency of biguanide action is found, which might be brought about by the β-adrenergic system based on a molecular interaction to biological membranes [10].

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REFERENCES

1. N. Gyr, W. Berger, R. Fridrich, A. Denes and G. A. Stalder, *Schweiz. Med. Wschr.* **101**, 1876 (1971).
2. L. Biro, T. Banyasz, M. B. Kovacs and M. Bajor, *Klin. Wschr.* **39**, 760 (1961).
3. A. Czyzyk, J. Lawecki, J. Sadoswki, I. Ponikowska and Z. Szczezepanik, *Diabetes* **17**, 492 (1968).
4. M. Kessler, W. Meier, C. Storelli and G. Semenza, *Biochim. Biophys. Acta* **413**, 444 (1975).
5. R. A. Altschuld and F. A. Kruger, *Ann. N.Y. Acad. Sci.* **148**, 612 (1968).
6. R. Haeckel and H. Haeckel, *Konferenz der Gesellschaft für Biologische Chemie Bd* **351** (1970).
7. W. Losert, E. Schillinger, W. Kraaz, O. Loge and P. Jahn, Arzneim. Forsch. (Drug Res) **22**, 1540 (1972).
8. G. L. Searle and R. R. Cavalieri, *Ann. N.Y. Acad. Sci.* **148**, 734 (1968).
9. H. D. Söling and W. Creutzfeld, *Int. Biguanid-Symposium, Thieme Verlag* 17 (1960).
10. G. Schäfer, *Biochem. Pharmac.* **25**, 2005 (1976).
11. A. Gulbenkian, M. Grasso and T. Scull, *Endocrinology* **92**, 326 (1973).
12. A. Loubatières, M. M. Mariani and F. Jallet, *C.R. Acad. Sci., Paris* **272**, 335 (1971).
13. E. E. Ohnhaus and W. Berger, *Diabetologia* **11**, 367 (1975).
14. J. Grayson, *J. Physiol.* **118**, 54 (1952).
15. E. E. Ohnhaus and E. Hunziker, *Pflügers Arch.* **347**, 255 (1974).
16. J. Grayson and D. H. Johnson, *J. Physiol.* **120**, 73 (1953).
17. E. E. Ohnhaus, E. Emons and A. Breckenridge, *Pflügers Arch.* **323**, 182 (1971).
18. F. M. Schmidt, *Klin. Wschr.* **39**, 1244 (1961).

19. C. N. Hales and P. J. Randle, *Biochem. J.* **88,** 137 (1963).
20. T. Sachs, *Statistische Auswertungsmethoden.* Springer, Berlin (1969).
21. C. R. Morgan and A. Lazarow, *Diabetes* **14,** 669 (1965).
22. J. G. Kral, *Horm. Metab. Res.* **6,** 483 (1974).
23. D. Porte, Jr., A. Graber, T. J. Kuzuya and R. H. Williams, *J. clin. Invest.* **45,** 228 (1966).
24. C. V. Greenway and A. E. Lawson, *Can. J. Physiol. Pharmac.* **46,** 906 (1968).
25. A. Geumei and M. Mahfouz, *Br. J. Pharmac. Chemother.* **32,** 466 (1968).
26. C. V. Greenway and R. D. Stark, *Physiol. Rev.* **51,** 23 (1971).
27. W. Berger, *Aktuelle Probleme in der Angiologie, Bd.* **16,** Hans Huber Verlag, Bern (1972).