TONE AND REACTIVITY OF VASCULAR SMOOTH MUSCLE IN GERMFREE RAT MESENTERY*

By SILVIO BAEZ, M.D., and HELMUT A. GORDON, M.D.

(From the Departments of Anesthesiology and Physiology, Albert Einstein College of Medicine, New York 10461, and the Department of Pharmacology, College of Medicine, University of Kentucky, Lexington, Kentucky 40506.)

(Received for publication 1 June 1971)

Rodents reared under germfree conditions exhibit cardiovascular and hemodynamic features which are at variance from similar animals reared in open animal-colony environment.

When compared to conventional counterparts, germfree rats, in addition to an indication of smaller heart size and lower blood volume, show significantly lower cardiac output (1) and a relatively diminished regional (liver, intestine) blood flow (2). In spite of such differing circulatory morphology and physiology in earlier separate studies, investigators were unable to disclose any significant differences in survival rate of germfree and conventional rats after similar standard stress of hemorrhagic hypotension (3–6). Similarly, no difference in survival rate was noted when the hemorrhagic shock stress was imposed subsequent to cecectomy in germfree and conventional rats (7). The published data shows that systemic blood pressure in germfree rats has no compensatory rebound as is usually seen in controls. After each bleeding, the blood pressure in germfree rats tended to remain at its initial low level. In terms of pathology, after transfusion of the shed blood and irreversible shock, the liver appeared small, pale, and somewhat contracted in the majority of germfree rats. The liver was dark, congested, and distended in conventional control animals (3). A more recent report (8), however, shows a clear-cut greater survival rate of germfree rats as compared to conventional controls, both before and after cecectomy, after similar standard hemorrhagic shock procedures. The better survival figures in germfree rats occurred in a phase of significantly longer time to achieve maximum blood loss and a lesser uptake of the shed blood. No explanation, however, is forthcoming to account for the differing hemodynamic adjustment and final outcome.

Although the germfree rodent sustains normal growth, reproduction, and life-span (9), it possesses an exaggerated atonic enlargement of the cecum (10) and studies show the presence of active substances in the cecal content of germfree rats and mice (11). Some of these substances strongly affect the tone of plain muscle in a number of preparations (12–14). It is therefore possible that the lack of systemic blood pressure response after blood loss in germfree rats is related to modification in the tone level and reactivity of smooth muscle in the wall of precapillary arterioles.

* This work was supported in parts by the National Institutes of Health, U.S. Public Health Service Grants No. HE-06736-10, GM-13661-03, and AM-14621-04.

The purpose of this investigation was to examine and compare the responses of muscular precapillary vessels to selected vasoactive drugs in germfree and conventional rats. Part of the results have been presented in a preliminary communication (14).

Materials and Methods

All of the rats used in these experiments were of the same strain (Fischer 344, Charles River, C.D.F. [Charles River Breeding Laboratories, Wilmington, Mass.]) of both sexes weighing 140–160 g each. They were kept on steam-sterilized L-462 diet (Lobund Institute, Notre Dame, Ind.) (15) fed *ad libitum*. The germfree rats were maintained in flexible plastic isolators (16), and the conventional controls in the open environment of the same air-conditioned room. The day before the experiment the animals were fasted overnight with free access to water. The germfree rats remained in the isolators until the injection of minimal anesthetic (pentobarbital sodium 25 mg/kg) required for surgery, at which time they were exposed to the laboratory environment. A tracheostomy was performed to insure a clear airway, and a carotid artery was cannulated for continuous monitoring of the systemic blood pressure. The cecum was carefully exposed and placed upon a specially built platform attached to the stage of a microscope. Except for a multiple (four) outlet irrigation source required to keep moisture and temperature of the oversized cecum, and the use of an adequate lucite block to receive the corresponding mesoappendix, all other procedures for the in vivo study of the microcirculation in this tissue were adhered to as previously described (17). From the moment of exposure, cecum, ileum, and attached mesentery tissues were moistened by a continuous drip of mammalian Ringer's solution (37.5 $\pm$ 0.5°C) of the following composition in mM/liter): NaCl, 154.3; KCl, 5.63; $CaCl_2$, 2.16; gelatin (10 mg/liter), adjusted to pH 7.4 with $NaHCO_3$. In all experiments, a 15 min period was allowed for the stabilization of the preparation before starting measurements and drug stimulation.

Blood pressure was monitored with a PG-10 Statham transducer (Statham Instruments, Inc., Los Angeles, Calif.) and a Grass No. 7 polygraph (Grass Instrument Company, Quincy, Mass.). Observation and microphotography of the microcirculatory bed was made at $\times$ 120 optical magnification using a Bausch & Lomb triocular microscope (Bausch & Lomb Inc., Rochester, N.Y.). Measurements of vessel dimensions and its changes were made at $\times$ 3500 magnification on the television screen using the image-shearing method already described (18).

Before application of the stimulating agents, several accurate simultaneous measurements of vessel inner and outer radii, and wall thickness were made at three levels of precapillary arterioles: large, primary arteriole (A),[1] i.e. vessel arising from the terminal artery; secondary arteriole (A′) resulting from bifurcation of the former; and metarteriole (M) which arises from either of the two former vessels. A target arteriole is then randomly selected for evaluating the response to the stimulating drug by measuring changes in lumen diameter alone.

The smooth muscle-stimulating drugs employed were the catecholamines epinephrine (adrenaline chloride; Parke, Davis & Co., Detroit, Mich.) and l-norepinephrine (levarterenol bitartrate, Levophed; Winthrop Laboratories, New York), diluted stock solution (100 μg/ml) with distilled water, and the polypeptides angiotensin amide (Hypertensin; CIBA Pharmaceutical Company, Summit, N.J.) and vasopressin (Pitressin; Parke, Davis & Co.) also diluted with distilled water in stock solution of 10 μg/ml and 1.0 IU/ml, respectively. Further dilutions of the stimulants were made as needed with warm Ringer's solution just before topical application in a 0.05 ml volume. Inasmuch as such a small volume of the drop solution was

[1] *Abbreviations used in this paper:* A, primary arteriole; A′, secondary arteriole; angiot, angiotensin; 1:∞, lumen:wall ratio; M, metarteriole; norepi, norepinephrine; pitress, Pitressin; ri, inner radius; ro, outer radius; w, wall thickness.

delivered along the perfusion fluid resulting in further dilution of an unknown extent, notation was made of the amount of the agents per milliliter.

The procedures were standardized as much as possible in all of the experiments and the observations and measurements were completed within 50–60 min from the moment of first exposure of the germfree animals to the laboratory environment. The following symbols were used to designate the measured vessel dimensions: ro, outer radius; ri, inner radius; w, wall thickness; and $l:\alpha$ = lumen:wall ratio.

RESULTS

In spite of disparate (three to four times) enlargement of the cecum, the mesoappendix lamella in germfree rats appears in general only slightly (one-

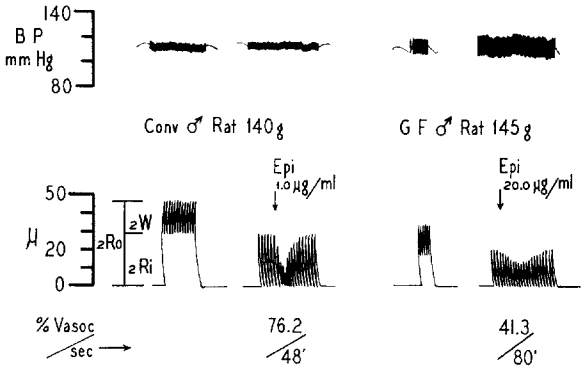

FIG. 1. Refractoriness of mesentery arteriole to epinephrine in the germ-free rat. *Top:* carotid artery blood pressure (BP). *Bottom:* 2 Ro, total vessel diameter; 2 Ri, lumen diameter; 2 W, wall thickness, respectively of an arteriole in conventional rat (conv), and of a metarteriole in germfree (GF) rat's mesentery. In the record under epinephrine (Epi) each deflection is a measure of the vessels lumen diameter at the time. % Vasoc, per cent vasoconstriction to epinephrine, sec, duration of the effect in seconds. Note the marked hyporesponsiveness of metarteriole in germfree to epinephrine (for further detail, see text).

half to one times) larger than corresponding tissue of conventional rats. Because the mesoappendix tissue in the former animals exhibited only scant development of fat cells, the observation and measurement of the microcirculatory pattern was considerably facilitated. Microscopic observation at × 120 magnification showed that except for a greater tortuosity of postcapillary collecting venules and veins, no other outstanding morphological features distinguished the germfree mesoappendix vasculature from that of conventional rats. An equal number of arterioles was found to arise from the terminal artery both in conventional and germfree mesoappendix. Due to the larger mesoappendix membrane in germfree animals, the tissue appeared relatively less vascularized as compared to the corresponding controls. A rapid unidirectional flow, present in arterioles and venules, compares well in the two sets of animal conditions.

One distinguishable characteristic in germfree mesoappendix vasculature, however, was the paucity in active vasomotion (Table III).

Hyporesponsiveness of Germfree Arterioles to Catecholamines.—The protocol (Fig. 1) shows typical examples of experiments designed to evaluate and compare this responsiveness of germfree and conventional rats' mesentery microvessels to selected vasoactive drugs. After several simultaneous measurements of radii and wall thickness, application of epinephrine (1.0 μg/ml) in the conventional rat (left, Fig. 1) resulted in a marked (peak 76.2%) narrowing of arteriolar lumen. In striking contrast to this, the germfree animal's (right, Fig. 1) vessel lumen decreased moderately (peak 41.3%) only when a much greater (20.0

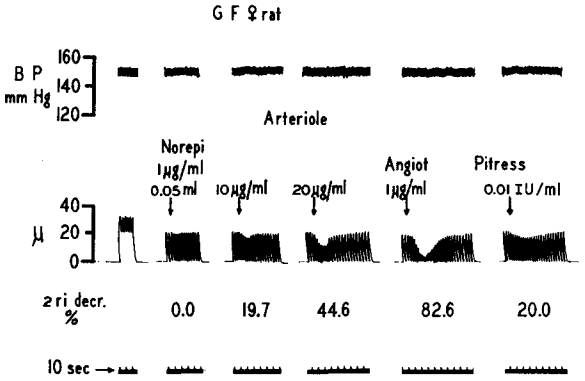

FIG. 2. Refractoriness of arteriole to norepinephrine (norepi) and Pitressin (pitress) in germfree (GF) rats. *Top:* carotid artery blood pressure (BP). In the *middle* record each deflection is the measure of the vessel lumen at the time. Arrows indicate the application of various smooth muscle agonists at 10 min intervals. The numbers in the *bottom* of the record are the magnitude of vessel lumen narrowing in per cent. Note hyporesponsiveness of the test mesentery arteriole to norepinephrine and vasopressin (Pitressin), but not to angiotensin (angiot) (for further description, see text).

μg/ml) dose of the drug was applied. It is of interest to note the longer time course (80 sec) of vessel response in the germfree rat than in the conventional rat (42 sec). Also the different size of vessels employed, a primary arteriole (30 μ lumen) in the conventional rat and in the germfree a secondary arteriole (20 μ lumen), is of importance. Customarily, in rodents reared in open laboratory environment, the sensitivity of precapillary microvessels to drug stimuli is inversely proportional to their lumen:wall ratio. The lesser the lumen:wall ratio of precapillary vessels, the greater its sensitivity of vasomotor stimuli (19).

The protocol (Fig. 2) shows another example of experiments in which, in addition to norepinephrine, the effect of angiotensin and Pitressin was examined in arterioles of the same germfree rat's mesentery preparation. In the contractile record, as expected, a moderate (peak 44.6%) narrowing of vessel

lumen (2 ri) occurred only when 20 μg/ml solution of norepinephrine was applied. The application of angiotensin (1.0 μg/ml) and Pitressin (0.01 IU/ml) at 10 min intervals resulted in a marked (84.6%) lumen decrease with the former and only slight (20.0%) decrease for the latter.

The mean and standard deviation (SD) of measurements of these and other similar experiments in 34 germfree and 25 conventional rats is shown in Table I. Because the microvascular hyporesponsiveness to epinephrine and norepinephrine was found essentially similar, in the table, under norepinephrine, five germfree and four conventional animals tested with epinephrine are included.

TABLE I

*Comparative Response of Germfree and Conventional Rat's Arterioles to Norepinephrine, Angiotensin, and Pitressin**

Animal type	No. Exp.	B. wt.‡	BP‡	Vessel size	Drug dose§	V. constr.§	Durat.§
		g	*mm Hg*	*μ*	*(Norepinephrine) μg/ml*	*%*	*sec*
Conv‖	11	126 ± 9.5	118 ± 7.3	19.2 ± 1.1	1.2 ± 0.4	53.7 ± 11.2	75 ± 32.3
GF‖	17	121 ± 8.3	144 ± 12.7	21.5 ± 2.0	26.2 ± 15.0	39.7 ± 19.3	107 ± 28.3
					(Pitressin) IU/ml		
Conv	7	122 ± 6.3	124 ± 6.2	19.8 ± 0.8	0.001 ± 0.02	37.0 ± 19.4	68 ± 22.3
GF	9	120 ± 6.5	143 ± 9.2	21.0 ± 1.7	0.02 ± 0.03	31.0 ± 16.4	72 ± 32.6
					(Angiotensin) μg/ml		
Conv	8	119 ± 7.1	122 ± 9.5	20.0 ± 1.3	1.1 ± 0.8	47.2 ± 13.1	72 ± 22.0
GF	9	123 ± 6.7	146 ± 12.5	23.9 ± 3.5	1.0 ± 0.0	43.7 ± 15.2	81 ± 39.7

* All three vasoactive agents were delivered topically, 0.05 ml volume.

‡ B. wt., body weight; BP, blood pressure.

§ For each drug category, the mean and SD of dose employed, the induced vasoconstriction in per cent, and the duration of effect in seconds are given in the last three columns.

‖ Conv, conventional; GF, germ free.

As compared to conventional rats, a profound hyporesponsiveness of the mesentery precapillary vessels to these catecholamines was a consistent finding in germfree rats of both sexes. In order to produce an average 45% arteriolar lumen narrowing, a 25-fold greater amount of the catecholamines was required in germfree animals as compared to conventional controls (germfree, norepinephrine [norepi] = 26.2 μg/ml ± 15.0; control, norepi = 1.2 μg ± 0.4). Similar, but less dramatic, arteriolar hyporeactivity to Pitressin was present in germ free rats (germfree, Pitressin [pitress] = 0.02 IU/ml ± 0.03; control, pitress = 0.001 IU/ml ± 0.02). In contrast to these, no differences in vascular responses to angiotensin were seen between the two experimental groups (germfree, angiotensin [angiot] = 1.0 μg/ml ± 0.0; control, angiot = 1.1 μg/ml ± 0.8).

Microvessel Geometric Variables.—In 10 of the conventional and 8 germfree

rats, measurement of radii and wall thickness was made at comparable sites of precapillary vessels. In a typical example of such evaluations (Fig. 3), greater lumen size (2 ri) was measured at corresponding anatomical levels of precapil-

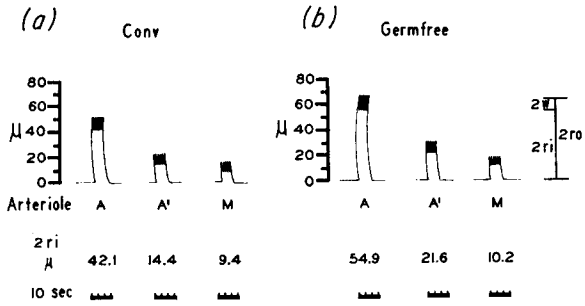

FIG. 3. Measurement of vessel dimensions of three similar precapillary vessels in mesentery of conventional (*a*) and germfree rats (*b*). A, primary arteriole, A′, secondary arteriole, M, metarteriole. Numbers in *bottom* of the record are the measured lumen diameter in microns. 2 ri, inside diameter, 2 ro, outside diameter; 2 W, wall thickness. Time marker = 10 secs. Note that wall thickness compares well in the various vessels of conventional and germfree, but a greater lumen size for all three vessels in the latter (for further detail, see text).

TABLE II

Comparative Dimensions of Similar Arterioles (A and A′) and Metarteriole (M) in Conventional and Germfree Rat Mesentery

Arteriole type*	No. measur.	Vessel geometry in microns			1/∝ ratio
		2 *ro*‡§	2 *ri*	w	
			(*Conventional*)		
A	40	43.2 ± 5.6	31.7 ± 6.9	5.8 ± 0.9	5.8 ± 2.4
A′	52	31.5 ± 2.8	21.3 ± 3.1	5.2 ± 1.3	4.2 ± 1.4
M	70	21.3 ± 2.7	11.0 ± 2.1	5.2 ± 1.0	2.2 ± 0.6
			(*Germfree*)		
A	24	66.8 ± 9.2	52.9 ± 8.4	5.7 ± 1.0	9.3 ± 1.9
A′	39	36.1 ± 5.1	25.2 ± 4.1	5.9 ± 0.9	4.7 ± 0.7
M	23	21.6 ± 4.6	12.9 ± 3.3	4.2 ± 0.8	3.0 ± 0.5

* A, primary arteriole, A′, secondary arteriole, M, metarteriole.

‡ 2 ro, outer diameter, 2 ri, inner diameter, 1/∝ = lumen to wall ratio.

§ The mean and SD respectively for total (2 ro) and inner (2 ri) diameters and wall thickness (w) are given in microns in the first three columns. The mean and SD of the ratio of lumen to wall for all vessels in each category is given in the last column.

lary arterioles (A, A_1) and the metarteriole (M) of the germfree rat than in conventional controls. The mean and standard deviation of the measured radii and wall thickness and the calculated l: ∝ ratios of all three types of precapillary vessels of germfree and conventional animals are included (Table II). The greater l: ∝ ratio of primary arteriole (A) in germfree rats (1/∝ = 9.3 ± 1.9)

than in conventional ($l/\propto = 5.8 \pm 2.4$) is related to the larger 2 ri in the former and not to differences in wall thickness (w).

DISCUSSION

The primary purpose of this investigation was to examine and compare the tone level and reactivity of the microvasculature in germfree rats and rats reared in the open laboratory environment. The results show that the mesentery microvessels and microcirculation in the germfree differs in a number of features from that of its conventional counterpart.

The enlarged (one-half to one times), relatively fat-free, mesoappendix of the germfree rat was found to be supplied by the same number of primary arterioles

TABLE III

Number of Supplying Primary Arteriole (A) and Rate of Vasomotion in Conventional and Germfree Mesoappendix Vasculatures

Animal type-No.	B. wt.	BP	Artl. (A) No.	Vasomotion	ETC*	Cap. flow‡
	g	*mm Hg*		*rate/min*	*epi. μg/ml*	
Conventional	128.0	118.0	5.0	1.0	1.2	Rapid
(11)	±8.5	±7.3	±0.08	±0.02	±0.4	
Germfree	125.0	132.2	5.0	0.4	25.2	Rapid
(12)	±7.2	±13.7	±0.06	±0.003	±11.0	

* ETC, epinephrine threshold concentration, i.e., amount of the stimulant required to produce an average 45% vasoconstriction.

‡ Cap. flow, general appearance of overall blood flow in the entire capillary bed in the field of observation.

as in the conventional control. The primary arteriole, and to a lesser extent secondary arterioles and the metarteriole, tends to exhibit greater mean lumen: wall ratios in the germfree rat (Table II). The fact that such gain in $l: \propto$ ratio of the microvessels resulted from an increase in inner and total diameter of the vessel without concomitant overt modification in wall thickness is of some interest in more than one respect: (*a*) It strongly suggests that the increment in microvessel lumen in the germfree animal is not an acute event, i.e., a consequence of the brief (50–60 min) exposure of the host animal to the open laboratory environment. It appears to be rather a slow chronic process. This view is buttressed by the fact that active dilatation of microvessels, either spontaneous or drug induced (20), or passive, by shift of internal pressure (21), invariably results in corresponding measurable changes in wall thickness. (*b*) Lumen increase in the absence of thinning out of the wall of the resistant microvessels may be germane to the reported (3) consistently higher level of mean systemic arterial blood pressure seen in germfree animals (Table III and reference 3) and will be given further consideration later in this discussion.

As compared to the microvasculature of conventional animals, similar vessels in germfree rats show a marked decreased vasomotor activity at all levels of precapillary arterioles and the precapillary sphincters. Direct counting of spontaneous activity in arterioles A (Table III) shows that the rate of vasomotion in the germfree is reduced by more than 100% in comparison to the control group. In addition, the cycle of activity, constriction–dilatation, is definitely of longer duration in the germfree vasculature. Either one sees at the onset of observations an occasionally closing precapillary sphincter which remained closed for as long as 40–60 min (the duration of the experiment), or initially a nonperfusing capillary started perfusion and remained in the circulation throughout the period of observation. A direct consequence of the reduced precapillary vasomotor activity was subjectively reflected in the microcirculatory pattern of germfree rats' mesentery, in which a continuous unidirectional, quiescent, blood flow prevailed through perfusing capillaries, tortuous venules, and collecting veins. In contrast to this, the microcirculation of conventional rats, with an unmodified rate of vasomotion, shows a rapid blood flow, periodically alternating from one capillary to another.

The most striking difference, however, between the mesentery vasculature of germfree and conventional animals was the marked microvascular refractoriness in the former animal condition to at least three of the four vascular smooth muscle agonists employed, i.e., the catecholamines, epinephrine and norepinephrine, and the polypeptide, vasopressin. It is of interest, however, that the response of similar precapillary vessels to angiotensin was essentially similar in both groups of animals. Such refractoriness of the microvessel effector cell to selected smooth muscle agonists but not to others is of considerable relevance in regard to the observed (Table III), and previously reported by others (3), slight but consistently higher values of systemic blood pressure in the germfree rodent than of conventional control. It is entirely possible that the effector cell in the wall of pre- and postcapillary vessels of this and other tissues in germfree rodents might be even more receptive to other naturally occurring musculoactive molecules and biogenic mediators.

The modification in the tone level and reactivity of the local microvasculature in the mesentery membrane adjoining the enlarged cecum of the germfree animal is not surprising. Evidence is accruing which indicates that in the cecal lumina a number of active substances accumulates (10). One of these substances, i.e. "alpha pigment," in germfree cecal supernatant proved to have biochemical and biological characteristics similar to ferritin and apoferritin (11–13). The catecholamine-inhibitory action of ferritin and the carrier protein moiety apoferritin on microvascular smooth muscle is on record (22, 23). It will suffice to note that the increased protracted presence of such substances in the cecum and liver (24) of the germfree rat may per se account for the microvascular characteristics noted in the mesentery of the gnotobiotic rodent. It is of interest in this regard to note that the tone of germfree cecal muscle strips was found to

be reduced two-thirds that of conventional controls (25), and also to be less responsive to a number of musculoactive naturally occurring chemicals, including epinephrine, acetylcholine, histamine, and 5-HT (serotonin creatinine sulphate; Sandoz Pharmaceutical, Hanover, N.J.) (26).

Although the microcirculatory data described were derived from observations and measurements in the mesoappendix, it would appear safe to assume that the vascular characteristics disclosed, in germfree life, may not be restricted to this tissue and likely to be extended to microvasculatures of neighboring tissues and organs, e.g., terminal ileum and particularly cecal mural microcirculation. Should such microvascular modifications be so extended, it indeed would serve as a reasonable basis to account for the lag in achieving maximal bleeding time, and the lesser bleeding and uptake values during standard hemorrhagic experiments in these animals (8). It would also explain the observed paucity in systemic blood pressure rebound upon blood loss in the gnotobiotic rat (3). It is of interest to note in this context the fact that the hypotonic appearance and the refractoriness to catecholamines in the microvascular bed observed, in germfree animals, show many of the attributes of similar vascular beds of control animals injected with alpha receptor-blocking drugs, e.g., phenoxybenzamine (Dibenzyline; Smith Kline & French Laboratories, Philadelphia, Pa.), and which upon blood loss show comparable hemodynamic adjustments as in germfree animals, and higher survival rates than untreated controls (27).

The present study offers no sufficient basis to explain the noted marked better survival rate in the hemorrhagic shock-exposed germfree cecectomized animal than both the noncecectomized germfree and conventional control (8). It is known that surgical removal of the large cecum in germfree rats results in reversal of some of the modified cardiovascular and metabolic parameters of germfree life toward values similar to conventional rats (28), and that the cecal wall vasculature is an area of blood pooling in shock (3). The fact that better outcome, from the standard hemorrhagic stress in germfree cecectomized animals, was accompanied by a prolonged delay in maximal bleeding time, lesser bleeding volume, and minimal uptake of the shed blood from the reservoir, would strongly suggest that the modified microcirculatory parameters described may still persist subsequent to cecectomy. A germfree individual, endowed with a hypotonic, catecholamine-resistant microcirculation and relieved from the burden of the enlarged cecum, may be better off than conventional animals to withstand the circulatory stress imposed. Considerably more work is needed, however, in particular regarding the time of appearance and extent of the microcirculatory changes noted in the germfree individual. Also, the information regarding the changes, if any, of the microcirculation after cecectomy in the germfree rodent are needed to explain not only problems of shock, but those related to many of the modifications described in germfree life.

SUMMARY

Microcirculatory observations and measurements were made by in vivo microscopy in 35 germfree and 26 conventional rats. The rate of vasomotor activity, "vasomotion," of precapillary arterioles was found markedly decreased in the germfree animals.

All precapillary vessels in the germfree rats were markedly hyporeactive to the catecholamines, epinephrine and norepinephrine, as compared to similar vessels of conventional rats. The vessels in the germfree animals were also less responsive to vasopressin but not angiotensin.

A greater lumen:wall ratio of primary arterioles, but not of secondary arterioles and metarterioles, found in germfree animals is related to change in vessel lumen alone without concomitant change in wall thickness.

The germfree rat is characterized by possessing a hypotonic, catecholamine-refractory mesoappendix microvasculature.

The authors wish to thank Mrs. Zona Laidlaw, Miss Mary Miller, Mr. Larry A. Barton, and Mr. Carl Hatton for their excellent technical assistance.

BIBLIOGRAPHY

1. Gordon, H. A., B. S. Wostmann, and E. Bruckner-Kardoss. 1963. Effects of microbial flora on cardiac output and other elements of blood circulation. *Proc. Soc. Exp. Biol. Med.* **114:**301.
2. Wostmann, B. S., P. L. Knight, L. L. Keeley, and D. Kan. 1963. Metabolism and function of thiamine and naphthoquinones in germfree and conventional rats. *Fed. Proc.* **22:**120.
3. Zweifach, B. W., H. A. Gordon, M. Wagner, and J. A. Reyniers. 1958. Irreversible hemorrhagic shock in germfree rats. *J. Exp. Med.* **107:**437.
4. Zweifach, B. W. 1969. Hemorrhagic shock in germfree rats. *Ann. N.Y. Acad. Sci.* **78:**315.
5. McNulty, W. P., Jr., and R. Linares. 1960. Hemorrhagic shock of germfree rats. *Amer. J. Physiol.* **198:**141.
6. Heneghan, J. B. 1968. Hemorrhagic shock in unanesthetized gnotobiotic rats. *In* Advances in Germfree Research and Gnotobiology, International Symposium on Life-free Research. M. Miyakawa and T. D. Luckey, editors. Chemical Rubber Co. Press, Cleveland, Ohio. 166.
7. Gordon, H. A., E. Bruckner-Kardoss, T. Staley, E. Wagner, and B. S. Wostmann. 1966. Characteristics of the germfree rat. *Acta Anat.* **64:**367.
8. Heneghan, J. B., M. Peuler, A. Costrini, and I. Cohn, Jr. 1970. Hemorrhagic shock in cecectomized germfree rats. *Surg. Forum.* **21:**232.
9. Pleasants, J. R. 1967. Characteristics of the germ-free animal. *In* The Germfree Animal in Research. M. E. Coates, editor. Academic Press Inc., New York. 113.
10. Gordon, H. A. 1965. Demonstration of a bioactive substance in the caecal contents of the germfree animals. *Nature* (*London*). **205:**571.

11. Gordon, H. A. 1967. A substance acting on smooth muscle in intestinal contents of germfree animals. *Ann. N.Y. Acad. Sci.* **147:**83.
12. Kokas, E., and H. A. Gordon. 1966. Effect of bioactive substances in cecal content of germfree animals on spontaneous villous motility. *Fed. Proc.* **25:**199.
13. Gordon, H. A., and E. Kokas. 1968. A bioactive pigment ("alpha pigment") in cecal content of germfree animals. *Biochem. Pharmacol.* **17:**2333.
14. Baez, S., and H. A. Gordon. 1967. Microvascular refractoriness to epinephrine in germfree rats. *In* Proceedings of Symposium of Gnotobiotic Research, University of Wisconsin, Madison, Wis. 34.
15. Wostmann, B. S. 1959. Nutrition of the germfree mammal. *Ann. N. Y. Acad. Sci.* **78:**175.
16. Trexler, P. C., and L. I. Reynolds. 1958. Flexible film apparatus for the rearing and use of germfree animals. *Appl. Microbiol.* **5:**406.
17. Zweifach, B. W., and D. B. Metz. 1956. Rat mesoappendix procedure for bioassay of humoral substances acting on peripheral blood vessels. *Ergeb. Anat. Entwicklungsgesch.* **35:**181.
18. Baez, S. 1966. Recording of microvascular dimensions with an image-splitter television microscope. *J. Appl. Physiol.* **211:**299.
19. Baez, S. 1961. Response characteristics of perfused microvessels to pressure and vasoactive stimuli. *Angiology.* **12:**452.
20. Baez, S. 1969. Simultaneous measurements of radii and wall thickness of microvessels in the anesthetized rat. *Circ. Res.* **25:**315.
21. Baez, S. 1968. Bayliss response in the microcirculation. *Fed. Proc.* **27:**1410.
22. Shorr, E., B. W. Zweifach, R. F. Furchgott, and S. Baez. 1951. Hepatorenal factors in circulatory homeostasis. IV. Tissue origin of vasotropic principles, VEM and VDM, which appear during evolution of hemorrhagic and tourniquet shock. *Circulation.* **3:**42.
23. Shorr, E., A. Mazur, and S. Baez. 1955. Chemical and biological properties of hepatorenal factors VEM and VDM (ferritin). *In* Recent Progress in Hormone Research. Academic Press Inc., New York. 453.
24. Reddy, B. S., B. S. Wostmann, and J. R. Pleasants. 1965. Iron, copper and manganese in germfree and conventional rats. *J. Nutr.* **86:**156.
25. Staley, T. E. 1968. Cited by H. A. Gordon. The Germ-Free Animal in Research. M. E. Coates, editor. Academic Press, Inc., New York. 131.
26. Strandberg, K., G. Sedvall, T. Midtvedt, and B. Gustafsson. 1966. Effect of some biologically active amines on the cecum wall of germfree rats. *Proc. Soc. Exp. Biol. Med.* **121:**699.
27. Zweifach, B. W., S. Baez, and E. Shorr. 1952. Effect of dibenamine on humoral and vascular changes in dogs subjected to hemorrhagic shock. *Fed. Proc.* **11:**177.
28. Wostmann, B. S., E. Bruckner-Kardoss, and P. L. Knight, Jr. 1968. Cecal enlargement, cardiac output, and O_2 consumption in germfree rats. *Proc. Soc. Exp. Biol. Med.* **128:**137.