were further categorized into the percentages of phagocytic PAM's containing one (27 percent), two (15 percent), three (11 percent), four (8 percent), five (7 percent), and more than five (32 percent) spheres. The average percentage of cells with one or more spheres measured on 41 normal rats was 28.2 ± 8.2 percent.

Since this percentage was lower than anticipated, we designed experiments to determine if all macrophages in the alveolar region were being exposed to spheres and if the spheres were in high enough concentration. Rats were again instilled in vivo with green fluorescent spheres for 2 hours. Samples were washed to remove nonphagocytized spheres and then incubated in vitro for 30 minutes with red fluorescent spheres 1.65 μm in diameter (*7*). Microscopic examination showed that 35 percent of the PAM's had both red and green spheres, more than 60 percent contained only red spheres, and less than 5 percent had no spheres. Thus there were PAM's within the lungs that had not phagocytized green spheres but were capable of phagocytizing red spheres in vitro. These results suggested that the instillation of 1×10^7 to 2×10^7 spheres was insufficient to distribute them to all macrophages within the lungs. To alleviate this problem, we killed the rats, surgically exposed the trachea, opened the thoracic cavity, and instilled 15 ml of green fluorescent spheres (5×10^7 per milliliter) into the lungs hydrostatically (25 cm H_2O) by way of the trachea. After 30 minutes the lungs were lavaged and the percentage of phagocytic cells was quantitated microscopically and by FCM analysis. The results showed that more than 90 percent of the cells were phagocytic.

Finally, to determine if microspheres were being phagocytized or instead were being nonspecifically bound to the cell surface, rat PAM's were exposed in vitro to 0, 0.1, 0.3, and 1.0 percent sodium azide and green fluorescent spheres. Experimental FCM data showed (Table 1) that 91 percent of PAM's that were not exposed to sodium azide had associated spheres, whereas only 6 to 7 percent had spheres associated with them when exposed to 0.3 to 1.0 percent sodium azide. The microscopically determined phagocytoses were within 5 percent of the FCM results. Decreased phagocytosis, induced by sodium azide, was also shown in terms of a reduction in the number of particles per cell. Thus, as determined by FCM, the percentage of PAM's containing one to five spheres increased, whereas the percentage containing more than five spheres decreased. These data show that, as the inhibitor concentration increased, the percentage of phagocytic PAM's decreased and those PAM's that were phagocytic had fewer spheres.

Although Sprague-Dawley rats were used in these studies, results may differ among rat strains and animal species. Since the microspheres contain surface carboxyl groups, they can be coated with opsonins, antibodies, or other chemicals for studying specific receptor-mediated phagocytosis (*8*). This technology has broad application for the rapid and accurate determination of phagocytosis by many cell systems, including the study of the effects of environmental toxicants.

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Serum from Monkeys with Histories of Fetal Wastage Causes Abnormalities in Cultured Rat Embryos

Abstract. *Rat embryos in the mid-head-fold stage were cultured on female monkey serum for 48 hours. On the basis of embryo response, the investigators, with no knowledge of the donors' reproductive histories, correctly identified the serum from two high-risk breeders among 18 rhesus monkeys and the serum from 12 of 14 high-risk breeders among 26 pig-tailed monkeys as teratogenic. Of the embryo response parameters examined, abnormality type and frequency were more closely correlated with donor reproductive histories than embryo protein content or somite number.*

The feasibility of evaluating the teratogenicity of serum by using cultured whole rat embryos was first demonstrated with serum samples taken from rats injected with cadmium chloride or cyclophosphamide (*1*). Depending on dosage and the amount of time between teratogen administration and blood collection, the serum had reproducible effects on embryo survival, abnormality type and frequency, and protein and DNA content. The possibility of extending this approach to studies of humans was supported by the observation that rat embryos can grow and develop for 48 hours on human serum when glucose is added (*2*). Furthermore, serum samples from patients undergoing cancer chemotherapy or taking anticonvulsant medication were found to be teratogenic (*2*). Still, the relevance of these observations to the outcome of actual pregnancies could only be inferred. We now report a relation between the reproductive histories of female monkeys and the teratogenicity of their serum.

Our original objective in using monkeys as serum donors was to introduce primate metabolism as part of a general teratogen-screening procedure involving rat embryo cultures. Serum from 18 female rhesus monkeys (*Macaca mulatta*) from the California Primate Research Center (University of California, Davis) was tested. The samples were prepared in accordance with the procedures used for human serum (*2*), including centrifugation immediately after withdrawal, heat inactivation, sterile filtration, and the addition of antibiotics, water (10 percent by volume), and glucose to a final concentration of 3 mg/ml. Seventy-five rat embryos at mid-head-fold stage (*1*) were cultured in this serum for 48 hours. Serum samples from 16 of the monkeys supported excellent embryo growth and development. Only one of the 75 embryos was considered abnormal, and the mean protein content per embryo was 124 ± 3 μg. Rat embryos were also cultured on serum taken from four of these monkeys at the start of menstruation or on days 8 or 20. All the embryos were morphologically normal and accumulat-

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ed comparable amounts of protein. Embryos grown on serum from two of the monkeys were abnormal and had a reduced protein content (93 ± 3 μg). It was then learned that these two monkeys, unlike the other 16, had failed to become pregnant in 2 years of regular breeding. Approximately 1 year later new serum samples from these two monkeys again failed to support normal development of rat embryos. More important, the embryos showed the same types of abnormalities as had appeared 1 year earlier. Embryos cultured on serum from one monkey had abnormalities like those in Fig. 1D; embryos grown in serum from the other monkey had abnormalities like those in Fig. 1E.

A group of monkeys (*Macaca nemestrina*) maintained at the Regional Primate Research Center (University of Washington, Seattle) for studies related to their poor reproductive performance (*3*) provided the opportunity to search for a possible relation between reproductive history and serum teratogenicity. On the basis of the results of at least three pregnancies per animal, these females have been divided into low- and high-risk breeders. To date, 45 low-risk and 42 high-risk breeders have been identified in the colony, with the low-risk group producing 90 percent live-born offspring compared to only 45 percent for the high-risk group.

Serum samples from 12 low-risk and 14 high-risk females were tested. Six of the low-risk and 11 of the high-risk animals were bled several times to determine the reproducibility of the results. All samples were coded and the reproductive histories of the donors were not disclosed until the embryo culture test was completed and evaluations of the embryo responses were made. On the basis of these evaluations, the respective serum samples were classified as teratogenic or nonteratogenic (Table 1). The serum classifications coincided with the reproductive histories of the donors for 8 of the 12 low-risk breeders and 12 of the 14 high-risk breeders ($P = .009$, one-tailed Fisher's exact test). The accuracy of the classifications of the initial samples (77 percent) was unchanged when the results for repeated samples were included (13 of 17 animals yielded two or more samples with the same classification).

Five morphological types, one normal (Fig. 1A) and four abnormal (Fig. 1, B to E), were recognized among embryos cultured for 48 hours on serum from low- or high-risk breeders. Abnormalities appeared in 72 percent of embryos cultured on serum from high-risk breeders and in 29 percent of embryos grown on serum from low-risk breeders. In the latter embryos the most frequent abnormalities were a collapsed neural tube, anomalous

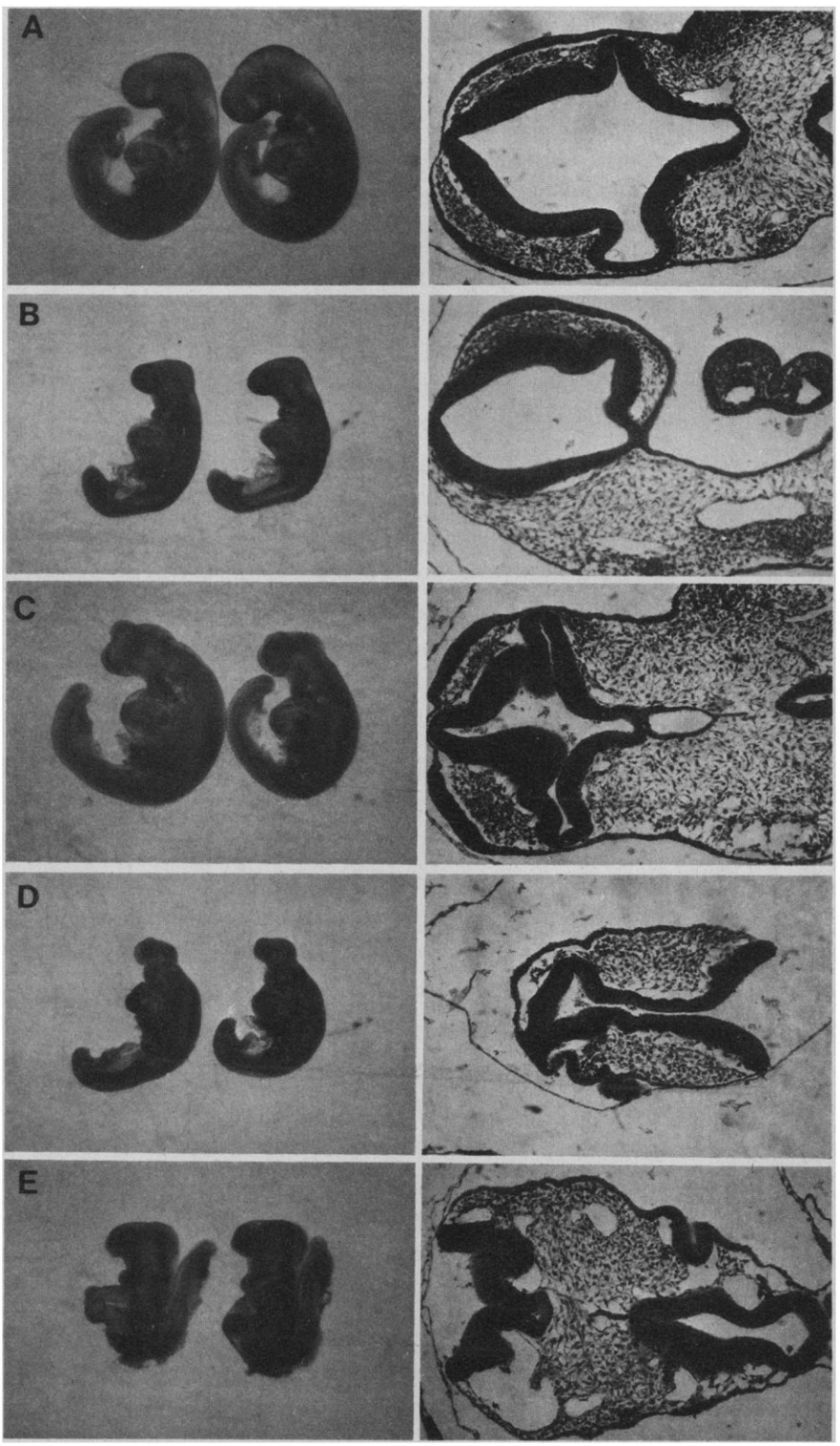

Fig. 1. Rat embryos after 48 hours of culture on serum from female monkeys with or without a history of fetal wastage. The photographs on the left (×24) are of live embryos immediately after removal of the extraembryonic membranes. The photographs on the right (×100) are of sections of embryos that were placed in Carnoy's fixative with membranes intact. Paraffin sections (7 μm) were cut and stained with Ehrlich's hematoxylin and periodic acid–Schiff reagent. (A) Normal embryos. (B) Morphologically disproportionate embryos with retarded eye development. (C) Embryos with collapsed neural tubes, abnormal folding of the neural epithelium, and retarded eye development. (D) Embryos with microcephaly, exencephaly, and anophthalmia. (E) Embryos with exencephaly, dorsiflexion of the trunk (with neural tube fusion), and anophthalmia.

folding of the neural epithelium, and retarded eye development. Microcephaly, exencephaly, and anophthalmia were the most frequent abnormalities associated with serum from the high-risk breeders. Although serum from some monkeys appeared to induce a particular morphological type, there was no relation between type of abnormality and type of reproductive dysfunction in the donor. (In Table 1, high-risk breeders M68018 through 74273 have histories of spontaneous abortion while the remainder show a mixture of fetal wastage problems.) Nevertheless, the most severe abnormalities (Fig. 1, D and E) were induced by serum from 12 of the 14 high-risk animals; these abnormalities were induced by serum from only three of the 12 low-risk breeders ($P = .005$). Embryo protein contents varied with morphological type, and the size difference between embryos cultured on serum from low-risk animals and embryos cultured on serum from high-risk animals was not significant. Similarly, somite numbers did not differ significantly, indicating that the differentiation of new somites may occur independent of embryo growth and other aspects of morphological development (*4*). All embryos used in this study had beating hearts at the end of the 48-hour culture period and were therefore considered alive.

Morphological development of the embryos was found to be more closely correlated with the reproductive history of the serum donor ($r = .64$, $P < .01$) than embryo protein content ($r = .28$, $P > .05$) or somite number ($r = .30$, $P > .05$). The importance of morphological development is also apparent from the results of the initial serum classifications. Two of the four serum samples incorrectly judged teratogenic (M71191 and 73446 in Table 1) were incorrectly evaluated because emphasis was placed on the small size of the embryos rather than the frequency of normal embryos (two-thirds of the embryos were morphologically normal). Plausible explanations for the other two of these errors include the presence of a temporary teratogen, such as an infectious agent, or particular sensitivity of these two monkeys to stress while being bled. Excessive handling of low-risk female monkeys greatly decreases the survival chances of their offspring (*5*), and lower rates of stillbirths have been reported for free-ranging monkeys (2.7 percent) than for cage-bred monkeys (15 percent) or pregnant animals captured in their native

Table 1. Growth and development of rat embryos cultured for 48 hours on serum samples from *Macaca nemestrina* females with histories of infrequent or frequent fetal wastage. Rat embryos (CD strain) were isolated after 9.5 days (the morning on which sperm was found in the vaginal smear was considered 0.5 day) and cultured with the yolk sac and ectoplacental cone intact but with Reichert's membrane removed (*2, 8*). Three embryos were cultured in 2 ml of medium and rotated at 30 rev/min in a room maintained at 37.5°C. At the start and after 4 hours the atmosphere was 5 percent O_2, 5 percent CO_2, and 90 percent N_2; after 20 hours, 15 percent O_2, 5 percent CO_2, and 80 percent N_2; after 27 hours, 30 percent O_2, 5 percent CO_2, and 65 percent N_2; and after 44 hours, 40 percent O_2, 5 percent CO_2, and 55 percent N_2. For protein determinations, individual embryos without extraembryonic membranes were dissolved in 1 ml of 1*N* NaOH and duplicate 0.2-ml portions were assayed by the method of Lowry *et al.* (*9*). Abbreviations: AB, abortion before day 130 of the 170-day gestation period; SB, stillbirth of fetuses at least 131 gestational days old; ND, neonatal death of live-borns from natural causes within 30 days of birth; NS, neonatal survivors (beyond 30 days); N, nonteratogenic; T, teratogenic. Numbers in parentheses indicate number of determinations used in statistical calculations.

Serum donor*	Number of each type of pregnancy outcome				Initial evaluation of each serum sample†	Morphological types induced by serum‡					Protein (micrograms per embryo)	Somites (pairs per embryo)
	AB	SB	ND	NS		A	B	C	D	E		
Low-risk												
M66291	0	0	0	4	N,N,N,N§	11		1			120.9 (12)	21.0 (10)
M71128	0	0	0	4	N,N	5					122.6 (5)	20.0 (5)
T71182	0	0	0	5	N	2			1		101.8 (2)	19.5 (2)
M71191	0	0	0	3	T	2	1				88.8 (2)	23.0 (1)
M72184	0	0	0	4	N,N	6					119.5 (5)	19.0 (5)
69491	1	0	0	4	T,T	2		3		1	86.4 (5)	15.6 (5)
72398	0	1	0	3	N	2		1			100.3 (3)	19.0 (1)
73446	1	0	0	3	T	2				1	69.6 (3)	21.0 (2)
73485	0	0	0	3	N§	5					107.3 (5)	20.0 (3)
74264	0	0	0	3	N	3					98.7 (3)	21.7 (3)
74278	0	0	0	3	N,T,N	5	3				97.3 (7)	18.3 (8)
74281	0	0	0	4	T,T			2	4		80.7 (5)	14.4 (5)
Total	2	1	0	43	N, 14; T, 7	45	4	7	5	2	99.5 ± 4.8¶ (12)	19.4 ± 0.7¶ (12)
High-risk												
M68018	2	1	0	1	N§	5					137.8 (5)	23.7 (3)
M72193	3	0	0	2	T,N	2	1		1	2	57.0 (5)	19.7 (3)
72045	4	0	0	1	T				3		118.0 (3)	20.0 (3)
72344	3	0	1	1	N,N,T	6		3			135.1 (8)	20.9 (8)
72413	2	1	0	1	T,T	1			3	2	70.7 (6)	17.5 (4)
73209	3	0	1	1	T,T		2		1	3	44.6 (5)	12.3 (3)
73481	3	1	0	2	T,T,T	2			6	1	87.1 (8)	18.3 (6)
73578	2	0	1	1	T,T				6		83.9 (4)	14.0 (5)
74273	3	1	0	0	T,T	1			5		71.6 (4)	15.5 (4)
M72058	1	2	1	0	T				3		91.5 (3)	17.7 (3)
M72094	1	0	2	0	T,T		3			3	73.0 (5)	19.0 (3)
71333	1	1	1	2	T,T					6	46.6 (4)	‖
71418	1	2	1	0	T,T,T	4	1		4		77.8 (8)	17.0 (8)
73184	1	1	2	1	T,N,T	4			5		96.4 (8)	17.8 (6)
Total	30	10	10	13	N, 5; T, 24	25	7	3	37	17	85.1 ± 7.8¶ (14)	17.9 ± 0.8¶ (13)

*Donors with alphanumeric designations were colony-born; the others were wild-born. The first two digits represent the year in which the animal entered the colony and the last three represent their order of entry in that year. †Evaluations were based on embryo morphology and size. Commas separate evaluations of samples drawn at least 1 month apart. ‡Types A to E correspond to the types shown in Fig. 1, A to E. §A single sample was tested twice; the evaluation was the same for both trials. ¶Mean ± standard error. ‖Embryos were too small for somites to be counted.

environment and brought to the United States (50 percent) (*6*). The two cases in which serum samples were incorrectly judged nonteratogenic may be explained by a disorder, such as an anatomical defect in a reproductive organ (*7*), that would not be expected to find expression in maternal serum.

Thus, rat embryos can be cultured successfully on monkey serum, and changes in the serum which might be expected to occur in relation to the menstrual cycle do not appear to adversely affect the serum's ability to support embryo growth and development. Furthermore, through evaluations of serum teratogenicity it was possible to correctly identify two high-risk breeders in a group of 18 rhesus monkeys, and 12 of 14 high-risk breeders in a group of 26 pig-tailed monkeys. To our knowledge this is the first observation of a relation between the biological effects of serum and the reproductive histories of the serum donors. These results would be expected if such factors as endocrine dysfunction, immunological incompatibility, nutritional deficiencies, and chronic infectious agents—implicated in human fetal wastage (*7*)—cause comparable problems in monkeys. Nevertheless, rat embryo cultures may be particularly suited for the detection of either serum factors that inhibit embryonic development or deficiencies of essential nutrients because they can grow and develop on high concentrations of serum (90 percent by volume or higher) and because they can be cultured during the period of rapid organogenesis, when development is particularly sensitive to interference.

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Reversal of Induced Ischemic Neurologic Deficit in Gerbils by the Opiate Antagonist Naloxone

Abstract. *Microsurgical unilateral occlusion of the right common carotid artery in 140 adult male gerbils produced homolateral cerebral ischemia and a neurologic deficit (stroke) in 42 percent (group A); the other 58 percent did not develop signs of stroke (group B). Intraperitoneal injection of the opiate antagonist naloxone (1 milligram per kilogram of body weight) reversed the signs of stroke within 3 to 5 minutes in ten out of ten group A gerbils; the effect lasted up to 30 minutes, after which stroke returned. Repeated injections of naloxone reversed stroke, but all ten gerbils died within 48 hours of ligation. However, nine other group A gerbils implanted with 10-milligram naloxone pellets had continuous reversal of signs of stroke, and four survived for more than 2 weeks. Twenty-one out of 24 group B gerbils injected intraperitoneally with morphine sulfate (5 to 30 milligrams per kilogram) 9 hours after ligation developed stroke within 3 to 20 minutes; morphine-induced stroke lasted 4 to 24 hours and could be reversed by intraperitoneal injection of naloxone. Ten out of 11 other group B gerbils injected intraperitoneally with the stereoisomeric opiate agonist levorphanol 9 hours after ligation developed signs of mild stroke that were reversed by intraperitoneal injection of naloxone. Ten other group B gerbils injected intraperitoneally with dextrorphan, the inactive enantiomer of levorphanol, 9 hours after ligation did not develop signs of stroke. Intraperitoneal injection of an enkephalin analog (Sandoz FK33824; 15 milligrams per kilogram) 9 hours after ligation did not produce stroke in ten other group B gerbils. These findings suggest the involvement of endorphins and opiate receptors in the pathophysiology of stroke and suggest the possible clinical use of opiate antagonists in humans in the acute phase of stroke.*

Recently, Brandt *et al.* (*1*) reported a hyperendorphin syndrome in a child with necrotizing encephalomyelopathy. After a protracted clinical course, the child lapsed into coma; intravenous administration of less than 1 mg of naloxone reversed his coma in four out of seven trials. High concentrations of endorphins were found in cerebrospinal fluid samples collected during the course of therapy and in the postmortem brain. As part of an ongoing experimental protocol designed to study the effects of neuropeptides on the function of the central nervous system, we recently found that hemiplegia secondary to cerebral ischemia in two patients could be reversed totally for up to 20 minutes by intravenous injection of 0.4 mg of naloxone (*1a*). Injection of placebo saline had no effect. Significantly, reversal of hemiplegia was not accompanied by any change in vital signs. Hemiplegia in both patients could be reversed repeatedly by intravenous injection of naloxone; in one patient, reversal of hemiplegia was obtained by naloxone injection over the course of several months. Moreover, in one of these patients, after the hemiplegia had resolved spontaneously, intravenous injection of morphine sulfate produced hemiplegia that was immediately reversed by intravenous injection of naloxone (0.4 mg). The hemiplegic condition of a third patient who had radiographically documented areas of cerebral infarction could not be reversed by intravenous injection of naloxone.

Because of this remarkable reversal of hemiplegia by naloxone, we studied the effects of naloxone on surgically induced ischemic neurologic deficit in gerbils that developed hemiparesis after occlusion of the right common carotid artery. We compared the opiate receptor binding capacity between the ischemic hemispheres and the contralateral hemisphere in these animals. We also studied the induction of stroke caused by the injection of morphine sulfate and the effects of stereoisomeric opiate agonists in gerbils that had not developed hemiplegia after occlusion of the common carotid artery.

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