Summary. When Walker carcinosarcoma 256 cells were grown under clonal growth technics, 50-100% plating efficiency was noted in a medium containing whole bovine serum. When dialyzed serum was used in the medium, few or no colonies were formed. Addition of pyruvate, α-ketoglutarate, and/or oxalacetate to the substrate resulted in plating efficiencies of 90-100%. Activity of these 3 metabolites was the same irrespective of addition of individual compounds or combinations thereof. Other Kreb's acids and metabolites tested failed to promote survival and growth of isolated Walker cells.

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Observations on Arteriovenous Communications in Lungs of Dogs.* (24026)

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The presence of precapillary arteriovenous communications in mammalian lungs has been one of the interesting questions of pulmonary vascular physiology. The study of Prinzmetal *et al.*(1) is often cited as evidence for these vascular communications. However, other workers(2,3) have suggested that precapillary shunts in the lungs are either very small or are possibly not extant at all. Because of the importance of these vascular passages, if present, in the economy of the pulmonary circulation, the following investigation was carried out.

Method. Mongrel dogs (8-14 kg) were anesthetized with sodium pentobarbital, and conventional Starling heart-lung preparations were set up. The arterial cannula was provided with a large side arm so that left ventricular output could be diverted to collecting vessels. Blood was heparinized. Pulmonary arterial pressure was recorded with a mercury manometer connected into the right upper lobar arterial branch. Cardiac output was adjusted to 400-500 ml/minute. Peripheral resistance was 80-90 mm Hg. Mean arterial pressure was usually 10-15 mm Hg. The preparations functioned without visible dilatation of the hearts. Lucite microspheres‡ in 2 g quantities were suspended in dog plasma colored with Evans blue dye and were rapidly introduced into the right atrium through stoppered venous cannula. Spheres of 75 μ and 250-300 μ were assorted by screening and agitation and the 2 sizes of spheres were used in all experiments since this quantity produced moderate rises in pulmonary arterial pressure. Two grams of 75 μ spheres comprise 1 million particles by count and 2 g of 250-300 μ particles number 500,000. After introduction of the plasma-sphere suspension, as the dye appeared in the arterial cannula, the cardiac output was diverted for 45 seconds through a stainless steel screen (325 mesh) to sieve out spheres which passed through the

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TABLE I. Results of Bead Injections in Conventional Heart-Lung Preparations.

Bead wt, g	Mean pulmonary art. press. mm Hg	Mean arterial blood pressure	Dog wt, kg	Beads recovered pulm. ven. blood	Size, μ
2	20 to 40	Decreased	14.5	0	75
2	20 50	"	11.3	+	"
1.75	5 40	"	10.2	+	"
1.75	10 45	"	10.4	+	"
1.5	10 30	"	12.7	+	"
2.5	20 45	"	10.9	0	300
1.9	5 20	"	14.5	0	"
2.5	20 45	"	8.0	0	"
2	20 40	"	9.0	0	"

lung vasculature. The atrial pressure was maintained at 15 cm of water by the addition of blood or saline to the venous reservoir. The screened spheres were identified by microscopic examination of the mesh.

Results are shown in Table I. In 4 out of 5 preparations in which beads of 75-80 μ were allowed to embolize the lungs, spheres were recovered from the pulmonary venous blood on the sieve. The retrieved particles represented about 0.5% of the total quantity introduced. On the other hand, in heart-lung preparations embolized with spheres of 250-300 μ, no particles were found on the collecting sieve. The consistent decline of systemic arterial pressure and elevation of pulmonary arterial tension after embolic assaults indicated that beads had reached small pulmonary arteries. After death of the preparations embolized with large spheres, no particles were found in the pulmonary veins, left cardiac chambers nor in the arterial cannula or tubing; however, spheres were readily identified throughout the pulmonary arteries.

Comment. From our data, there is evidence for the presence of pulmonary arteriovenous shunts in dogs, in that spheres of 75-80 μ appeared to pass through the lung vasculature under pressures engendered by the right ventricle. These data imply that the largest pulmonary arteriovenous connections are between 75 and 250 μ diameter. Since these heart-lung preparations are denervated it is possible that dilatation of any arteriovenous anastomoses had occurred maximally, thereby facilitating passage of the spheres.

Demonstrations of pulmonary precapillary AV shunts by embolic passage in our experiments and in those of others(1,5-8) are predicated on the assumption that pulmonary capillaries are in the order of 7 μ diameter(4), and that spheres of larger size can traverse the lung vasculature only through larger arteriovenous connections circumventing the capillary beds. Tobin and Zariquiey(5,6) injected suspensions of microspheres into pulmonary arteries at pressures up to 300 mm Hg, and identified particles of 500 μ in pulmonary venous blood. They suggested that the largest AV shunts were in lobar subdivisions of bronchopulmonary segments. Sersi and Bucher (7) concluded that 14.7% to 34.5% of radioactive particles (25-30 μ size) passed through AV communications to the pulmonary veins. Recently Niden and Aviado(2) noted the transit of spheres (60-420 μ) through the lung vasculature of dogs. Passage was increased by raising pulmonary perfusion pressure, or by the production of anoxia.

Experimental evidence of pulmonary AV shunts has not been unanimously accepted. Gordon, Flasher and Drury(9), using surface tension technics, noted that the largest AV connections in dog and rabbit lungs were about of 16 μ diameter. Knisley, Satterwhite and Wallace(10) reported their inability to recover spheres of 10 to 250 μ after injection at various sites into the veins, although in 18 of 36 animals small numbers of beads of 10-130 μ did traverse the lung vessels. They doubted the presence of precapillary anastomoses in normal lungs. They also suggested that recovery of beads in other experiments was due to escape of the particles into the vena cava and to the liver, or to excessive perfusion pressure forcing beads through capil-

laries or Thebesian veins thereby simulating AV transfers that may not exist.

Use of the heart-lung preparation precludes passage of microspheres into channels other than the pulmonary artery, and assures the propulsion of particles into the vasculature under physiological pressures. The recovery of spheres of 75-80 μ in the pulmonary venous blood leaves little doubt of the possibility of arteriovenous anastomoses in the pulmonary vasculature. Presumably these anastomotic connections function under conditions of increased pulmonary arterial pressure (such as occur in pulmonary embolism or heart failure) to spare the alveolar capillaries from overload. Other implied functions of these pulmonary AV shunts, as well as discrepant information as to their size, require further investigation.

Summary. Lucite spheres of 75-80 μ introduced into the pulmonary arteries of heart-lung preparations under physiologic pressures will pass from pulmonary arteries to veins. In contrast, microspheres of 300 μ size did not pass through the pulmonary vasculature.

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Influence of Varying Proportions in Combinations of Estrone, Estradiol and Estriol on Biological Response. (24027)

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Advances in analytical procedures, such as countercurrent distribution, as well as column and paper chromatography, have made it possible to identify and isolate estrogenic metabolites in relatively small (24-48 hour) collections of urine and in turn have stimulated much research. As a result, the interaction of these metabolites from a biological standpoint becomes even more intriguing. Hisaw and coworkers(1) and Wicks and Segal(2) stressed time and dose relationships. The concept of "impeded estrogens" was presented by Huggins and Jensen(3) and Edgren(4,5). Our interest in biological effectiveness of combinations of steroid hormones stems from 1948 when we reported results of androgenic mixtures on biological response(6). The present study was initiated in 1949.† Our aim was to see by what combination a synergistic effect could be realized or, if there was an inhibitory effect, at what level and in what combination it would be observed. From such data it may be possible to determine the type of biological activity that can be correlated with chemical assays, which determine presence and quantity of each estrogen in urine. A similar concept was postulated by Brown(7). Reports dealing with interaction of estrogen mixtures indicate that some workers employed either spayed mature(1) or hypophysectomized immature rats(3). Others employed intact immature mice(2,4,5). Other variables consisted of dose ranges, combination of estrogens and method of administering the mixtures.

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