TABLE I.
A Comparison of Lipids Found by Boyd's Extraction and in the Acetone Mother Liquors of Human Plasmas.
(Values are expressed in mg %.)

Sample	Total Lipid		Total Cholesterol		Phospholipid	
	Boyd	Acetone	Boyd	Acetone	Boyd	Acetone
1	527	378	145	71	149	133
2	471	323	123	72	136	113
3	642	498	159	114	193	167
4	528	392	135	94	159	155

would be much less—that is, using the acetone method but estimating the nitrogen of the precipitate rather than its weight. Lipid nitrogen is contained in the phospholipid fraction. It represents but a small part of the phospholipid fraction. The phospholipid fraction represents but one quarter of the total lipid. And phospholipids are more readily extracted by moist acetone (in the presence of other lipids and without magnesium chloride) than other lipids such as cholesterol.

The protein method of Bierry and Vivarro may be suitably modified to remove all of the lipids by simply diluting the plasma in a volume of acetone corresponding to the proportion of alcohol-ether used by Boyd.[2] It has been found that when plasma is diluted in 20 or 25 volumes of acetone, the acetone extract contains amounts of lipids identical to those in the alcohol-ether extract of the same sample of plasma. The suggested modification of the Bierry and Vivarro method as outlined by Peters and van Slyke[1] is thus to add 3 ml of plasma to 75 ml of acetone instead of 10 ml in the initial step of the procedure.

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Physiological Contraction of Double Hearts in Rabbit Embryos.

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The purpose of this paper is to show that the primitive lateral hearts in rabbit embryos are physiologically active and independent for some hours before they fuse into a single heart.

Knowledge concerning the early development of the mammalian heart has been obtained, for the most part, by the study of fixed

preparations. However, during recent years, several investigators have observed and reported the action of the hearts in living embryos in tissue culture.[1-4]

Embryos, the ages of which were calculated from observed matings, were obtained from a series of 12 rabbits. At the desired time the rabbit was given a moderate dose of nembutal, surgical anesthesia obtained by ether as needed, and the uterus removed. Embryos were cultured in hanging drops of adult rat plasma and 15-17-day rat embryo extract. A few embryos were observed immediately in buffered salt solution (Tyrode) before they were dissected away from the uterine wall.

Attempts to predict the stage of development of the embryo at a given time were only partially successful; about 50% of the rabbits were consistent, whereas the remaining 50% showed variation. However, 8 day 16 hour embryos were usually in the 4 or 5 somite stage. The hearts of these embryos were well formed and showed evidence of having been active for some hours. The 2 beating hearts were entirely independent of each other and were more widely separated that those observed in the rat by Goss.[3-4] The contraction originated at the venous end and both hearts beat at a regular rate, but the left was about 10 beats per minute faster than the right. The rate of the left heart varied from 47-49 beats per minute in the 3 somite stage, to about 80 per minute at the time of fusion of the 2 hearts (Table 1). The rate was correlated with the stage of development. In the earlier stages the ventricle was the only part of the heart involved in the contraction. A short time before the 2 hearts fused the atrium also showed contractions, in which case the impulse originated in the atrium.

Not only were the hearts beating at independent rates, but the type of contraction was surprisingly characteristic of each heart. The left side, which tends to be saccular, has a simple rapid "snap" contraction which originates in the venous end of the heart. The right heart tends to be of a tubular form, and the contraction is more complex. It may be described as a twisting wringing motion, similar to the 'peristaltoid' contraction described by Patten[5] in the chick. The time required to complete this contraction is longer than that of the left, so that the latent period between contractions is more nearly equal than one would expect from their respective rates.

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1 Waddington, C. H., and Waterman, A. J., *J. Anat.*, 1933, **67**, 355.

2 Nicholas, J. S., and Rudnick, D., *Proc. Nat. Acad. Sci.*, 1934, **20**, 656.

3 Goss, C. M., *J. Exp. Zool.*, 1935, **72**, 33.

4 Goss, C. M., *Anat. Rec.*, 1938, **70**, 505.

5 Patten, B. M., and Kramer, T. C., *Am. J. Anat.*, 1933, **53**, 349.

TABLE I.
Heart Rates in Cultured Rabbit Embryos.

No.	Age of embryo	No. of somites	Hours in culture	Heart rate		Remarks
				Left	Right	
W11B	8 days	3	2	49	37	Hearts widely
	12 hr		4	51	40	separated
			10	56	44	
G6A	8 days	4	2½	57	48	Hearts separated
	16 hr		4	58	49	" "
			10	60	48	" "
W12A	8 days	5	1½	66	38	" "
	15 hr		3½	65	38	" "
			9	68	43	" "
W10B	8 days	7	1½	82	79	" "
	15 hr		3½	82	80	" "
			9	63	63	" united
W10A	8 days	8	2	92	78	" separated
	15 hr		4	94	76	" "
			10	65	65	" united
Br8D	8 days	9	2½	86	78	" separated
	17 hr		5	89	77	" "
Br8E	8 days	11	3	88		Single heart
	17 hr		5	86		" "
			10	88		" "
Br8C	8 days	12	3	113		" "
	17 hr		5	86		" "

A backward and forward motion of cellular elements suspended in the plasma within the lumen of the vitelline veins was regularly observed in 7 somite embryos, and was occasionally seen at earlier stages. Both the vitelline veins and the aortae were connected to the hearts of their own side.

A similar movement has also been noted in the aortae at a slightly later stage in development. This movement is a pulsation caused by the contraction of the hearts, and though present on both sides, is more marked on the right side as would be expected because of the more vigorous type of contraction. There is a suggestion of approaching establishment of circulation, since the cellular elements do not always return to their original position within the aorta.

Two embryos developed single hearts after 10 hours in culture; these were 7 and an 8 somite embryos in which the hearts were widely separated when first observed. It was noted that in these hearts the impulse originated in the left atrium, as is the case in embryos with single hearts at the time of removal from the uterus.

The embryos were kept at a constant temperature of 38°, both during and between observations. They were not handled for one or 2 hours after being cultured, and then as little as possible. Recorded observations were made only after the culture had rested on the stage of the microscope for a few minutes. A few embryos have been observed in buffered salt solution (Tyrode) before removal from the uterine wall. These controls compared favorably with the observations made later in culture.

Summary. Rabbit embryos, removed during the ninth day of gestation, were cultured in hanging drops. Studies of the heart show that the 2 primitive lateral heart tubes beat regularly for some hours before they fuse to form one heart. There are marked differences between the 2 sides both morphologically and physiologically. The hearts develop considerably before fusing, as evidenced by the movement of cellular elements within the vessels. At early stages the contractions involve only the ventricles, but before fusion to form a single heart the impulses may be seen to originate in the respective atria.

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Andromimetic Effect of Estrogen upon the Clitoris of the Rat.*

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During the past 2 years, a number of female rats from an inbred strain of unrecorded origin have been found to possess spontaneous ovarian tumors which were invariably associated with defective external genitalia[1]. In order to interpret the physiology of these pathological ovaries, attempts were made to duplicate the abnormal external genitalia by the administration of sex hormones to normal females at different periods of postnatal life. It was observed that the genitalia, characteristic of the tumorous strain, could be experimentally produced by the injection of estrogen during the first week of postnatal life, but not if the treatments were begun thereafter. Since the studies on the ovarian tumor will be published subsequently, the present paper will be devoted to an interpretation of the effects of estrogen upon the external genitalia of the normal juvenile female rat.

It has been reported[2, 3] that the administration of estrogen to the

* The estrogen (Theelin) was supplied by Dr. Oliver Kamm, Parke, Davis and Company.

1 Turner, C. Donnell, *Anat. Rec.*, 1939, **73**, 75.

2 Hain, A. M., *Quart. J. Exp. Physiol.*, 1935, **25**, 131.

3 Greene, R. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 503.