

Resumen por los autores Bradley M. Patten y Rees Philpott.
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La contracción de los embriones durante los procesos preparatorios a la obtención de cortes.

Para averiguar el grado de contracción producido al fijar material embriológico y prepararle para la obtención de cortes, los autores han llevado a cabo varias series de medidas en embriones de cerdo durante los diversos momentos de algunos de los procedimientos comunes. Los fijadores usados fueron los siguientes: Licores de Zenker, Orth, y Tellyesnick; formol al 10%, formal-alcohol y licor de Bouin. Primero se midió la longitud del embrión desde la curvatura cefálica hasta la lumbar, cuando aquél estaba aún contenido en el líquido amniótico, y después a raíz del uso de cada una de las soluciones con que fué tratado. La contracción media fué medida en tantos por ciento de la longitud primitiva del embrión. Estas medidas fueron representadas gráficamente por curvas que indican el grado de contracción en cada uno de los momentos de la técnica empleada. La mayor contracción aparece después de la fijación con los líquidos que contienen bicromato, tales como los licores de Zenker, Orth y Tellyesnick, y la disminución de la longitud del embrión varía desde el 30% en embriones de 10 mm. hasta próximamente el 20% en los embriones de 20 a 25 mm. El grado mayor de contracción en los embriones más jóvenes debe probablemente atribuirse a la menor compacidad de su mesodermo. La mayor parte de la contracción en estos métodos es causada por los fijadores. La fijación en formol al 10% y en formol alcohol produce un hinchamiento inicial, al cual sigue una contracción rápida durante la deshidratación. La contracción total después de la inclusión en parafina es próximamente igual á la producida por las soluciones de bicromato. La contracción después del licor de Bouin es ligera, pero continúa durante la deshidratación e inclusión. La contracción total con este método es algo menor que la de los fijadores que contienen bicromato. La comparación de las diferentes gráficas indica una tendencia mayor hacia el aumento de la contracción durante la deshidratación después de los fijadores que producen menos contracción inicial. Esto prácticamente anula, por los menos en el caso del material destinado a la inclusión en parafina, las supuestas ventajas de los fijadores mezclados con el fin de evitar la contracción por el fijador mismo.

THE SHRINKAGE OF EMBRYOS IN THE PROCESSES PREPARATORY TO SECTIONING

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EIGHT FIGURES

INTRODUCTION

The true or fertilization age of the human embryos which come into the laboratory is usually uncertain if not altogether unknown. For the most part the age of other mammalian embryos used for laboratory study is equally a matter of conjecture. For this reason it is customary to designate the stage of development attained by an embryo by giving its measurements. In this country the method of measuring embryos used by Mall is in quite general use (Keibel and Mall, '10, vol. 1, ch. 8).

If embryos always came into the hands of the investigator fresh, so that the measurements might be made under uniform condition, the methods in use would leave little to be desired, at least as far as establishing a basis for comparing different embryos is concerned. Unfortunately, however, embryos are brought into the laboratory in various stages of preservation or lack of it. The shrinkage of embryos under the various treatments to which they are subjected for preservation and in preparation for sectioning makes the comparison of embryos measured at different steps in the processes a precarious matter.

While the fact that shrinkage is to be expected is a matter of common information, we have been unable to find in the literature any quantitative data covering the shrinkage which is to be encountered in the various processes of fixation commonly in use for embryological material. The measurements

described in this paper were made to ascertain how much shrinkage occurs in the various steps of some of the more common methods of preserving embryos and preparing them for sectioning. Because linear dimensions are already in use as a criterion of the stage of development attained, they were used, rather than weight or volume, as a basis for determining the shrinkage. It is hoped that these measurements and the further accumulation of similar data will aid in more accurate comparisons of embryos which come into the hands of investigators in widely different stages and conditions of preservation.

MATERIAL AND METHODS

By reason of the readiness with which they could be secured fresh, and because of their close comparability with young human embryos, pig embryos were used for the entire series of measurements.

The embryos were measured first in amniotic fluid,¹ immediately on removal from the uterus. The measurements made were for the crown-rump length. The belly thickness was also measured in quite an extensive series as a check against the possibility that distortion of the spinal axis might be affecting the length measurements. Inasmuch as the shrinkage in per cent of the original dimensions corresponded fairly closely in the two cases, only the crown-rump measurements are here recorded. All the measurements were made with micrometer calipers graduated in millimeters and indicating the tenths of a millimeter by vernier.

In the early part of the work both of us measured the same embryos independently and compared our results. Our measurements tallied so consistently to the tenth of a millimeter that in the latter part of the series measurements were made by only one observer.

After the first measurement in the amniotic fluid, embryos were measured after each solution with which they were treated.

¹ Some embryos were measured also in physiological saline solution and their measurements compared with those made in the amniotic fluid. The measurements in the two fluids were identical.

Following paraffin infiltration the embryos were transferred from the molten paraffin to xylol for measurement, after which they were at once returned to paraffin for a few minutes before embedding. This process proved to be in no way detrimental to the infiltration or embedding.

Measurements were later carried out on several series of sagittal sections made from the embryos measured during the preliminary processes of preparation. When care is used in expanding the sections on the slide, their measurements correspond to the measurements made subsequent to paraffin infiltration.

MEASUREMENTS

Since the processes to which the embryos were subjected, and therefore the series of measurements made on them, differ in accordance with the fixing fluid, the data secured may be most simply presented under the heading of the fixing agent used.

Zenker's fluid (formula as given in Lee, '13)

The technique employed was not different from that commonly used with Zenker's fluid: twenty-four hours in fixative; overnight washing in running water; about four hours in each grade of the ascending alcohols up to 70 per cent; twenty-four hours in 70 per cent alcohol containing Lugol's solution for the removal of any remaining mercuric chloride; overnight in 95 per cent alcohol; four hours in absolute (two changes); three hours in xylol; two to three hours in soft, and two to three hours in hard paraffin, according to the size of the embryos.

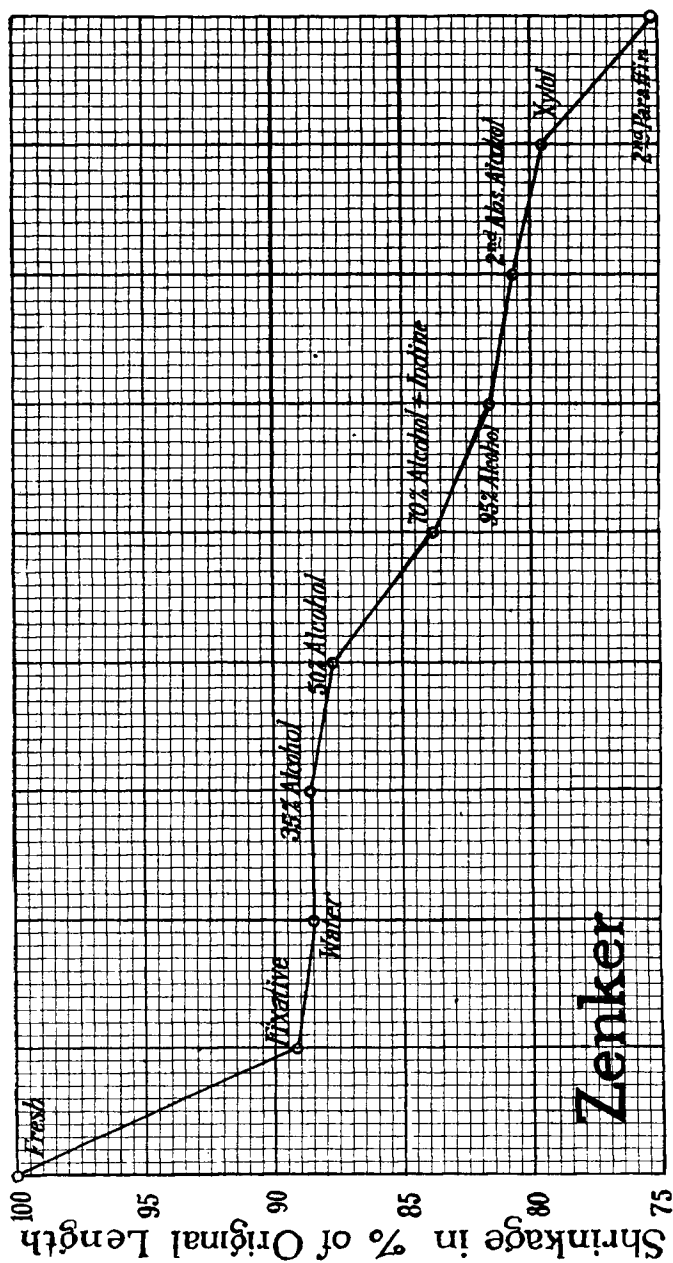
The measurements made on Zenker-fixed embryos are given in detail in table 1 and summarized graphically in the curve of figure 1. To facilitate comparisons for variability, the material in table 1 has been arranged in the order of the lengths of the embryos when measured fresh in the amniotic fluid. It will be seen that while there is, as would naturally be expected, some individual variability, the range of the variation is small. Indeed, comparison of the measurements made on embryos of like original

TABLE 1
Measurements of pig embryos, showing the amount of shrinkage which occurs in the various solutions used in the Zenker method of preparing material for sectioning

IDENTIFICATION NUMBER OF EMBRYO	C-R. LENGTH IN AMNIOTIC FLUID	ZENKER		WATER		35 PER CENT ALCOHOL		50 PER CENT ALCOHOL		70 PER CENT ALCOHOL AND LUGOL		95 PER CENT ALCOHOL		SECOND ABSOLUTE		XYLOL		SECOND PARAFFIN	
		C-R. length mm.	Shrinkage per cent	C-R. length mm.	Shrinkage per cent	C-R. length mm.	Shrinkage per cent	C-R. length mm.	Shrinkage per cent	C-R. length mm.	Shrinkage per cent	C-R. length mm.	Shrinkage per cent	C-R. length mm.	Shrinkage per cent	C-R. length mm.	Shrinkage per cent	C-R. length mm.	Shrinkage per cent
Z. a. 4	9.0	7.2	20.0	7.2	20.0	7.2	20.0	7.2	20.0	7.0	22.2	6.8	24.5	6.7	25.6	6.6	26.7	6.0	33.3
Z. a. 2	10.5	8.2	22.0	8.1	22.9	8.1	22.9	8.1	22.9	8.0	23.9	7.7	26.6	7.5	28.6	7.3	30.5	6.9	34.1
Z. a. 15	10.5	9.0	14.2	8.9	15.3	9.0	14.2	8.9	15.3	8.9	15.3	8.4	20.0	8.3	20.9	8.2	21.8	7.7	26.7
Z. a. 10	11.2	9.1	18.7	9.0	19.6	9.0	19.6	9.0	19.6	8.9	20.5	8.7	22.3	8.7	22.3	8.6	23.2	8.0	28.5
Z. a. 1	11.7	9.2	21.2	9.2	21.2	9.0	23.0	9.0	23.0	9.0	23.0	8.5	27.4	8.4	28.2	8.3	29.0	8.0	31.6
Z. a. 8	11.9	9.5	20.2	9.5	20.2	9.6	19.3	9.6	19.3	9.3	21.8	9.0	24.3	9.0	24.3	8.9	25.2	8.4	29.4
Z. a. 11	11.9	9.7	17.6	9.6	19.3	9.9	16.8	9.6	19.3	9.5	20.2	9.2	22.7	9.1	23.5	9.0	24.4	8.5	28.5
Z. a. 6	12.0	9.8	18.3	9.7	19.1	9.9	17.4	9.7	19.1	9.6	20.0	9.2	23.3	9.1	24.2	9.0	25.0	8.6	28.3
Z. a. 13	12.0	10.0	16.7	9.9	17.6	9.9	17.6	9.9	17.6	9.9	17.6	9.4	21.6	9.2	23.3	9.1	24.3	8.5	29.4
Z. a. 7	12.1	9.8	19.0	9.7	19.8	9.9	18.2	9.7	19.8	9.7	19.8	9.3	23.1	9.2	24.0	9.1	24.8	8.6	29.0
Z. a. 12	12.1	9.9	18.1	9.8	19.0	9.9	18.1	9.8	19.0	9.7	19.8	9.3	23.1	9.2	23.9	9.1	24.8	8.7	28.1
Z. a. 14	12.1	9.9	18.2	9.8	19.0	9.9	18.2	9.9	18.2	9.9	18.2	9.3	23.1	9.3	23.1	9.2	24.0	8.7	28.1
Z. a. 3	12.2	9.6	21.3	9.5	22.2	9.7	20.4	9.6	21.3	9.5	22.2	9.1	25.4	9.0	26.2	8.9	27.1	8.8	27.9
Z. a. 9	12.7	10.3	18.9	10.2	19.7	10.0	21.3	10.1	20.4	10.0	21.3	9.8	22.9	9.7	23.6	9.7	23.6	9.0	29.1
Z. a. 5	12.9	10.4	19.4	10.3	20.2	10.4	19.4	10.3	20.2	10.2	21.0	9.9	23.3	9.8	24.0	9.7	24.8	9.2	28.6
Z. c. 3	14.3	12.9	9.8	12.8	10.5	12.8	10.5	*	*	12.7	11.2	12.6	11.9	12.4	13.3	12.2	14.7	11.8	17.5
Z. c. 2	14.4	12.9	10.4	12.8	11.1	12.8	11.1	*	*	12.7	11.8	12.6	12.5	12.6	12.5	12.1	16.0	11.5	20.1
Z. c. 1	16.5	14.8	13.3	14.6	11.5	14.4	12.7	*	*	14.2	13.9	13.9	15.8	13.8	16.4	13.7	17.0	12.8	22.4

Z. c. 4	17.5	16.0	8.5	15.8	9.7	15.7	10.3	*	*	15.5	11.4	15.2	13.1	15.1	13.7	15.0	14.3	14.0	20.0
Z. c. 6	19.6	16.9	13.8	16.8	14.3	16.8	14.3	*	*	16.5	15.9	16.2	17.5	16.1	18.0	16.0	18.5	14.9	24.1
Z. b. 1	20.0	18.6	7.0	18.4	8.0	18.2	9.0	18.0	10.0	17.5	12.5	17.2	14.0	17.0	15.0	16.9	15.5	15.8	21.0
Z. b. 2	20.4	18.7	8.3	18.6	8.8	18.4	9.7	18.1	11.3	17.8	12.7	17.4	14.7	17.1	16.2	16.7	18.1	15.4	24.5
Z. c. 5	21.4	19.1	10.7	18.9	11.7	18.9	11.7	*	*	18.7	12.6	18.3	14.5	18.1	15.5	18.0	15.8	16.8	21.4
Z. c. 9	25.0	23.7	5.2	23.5	6.0	23.3	6.8	*	*	22.9	8.4	22.7	9.2	22.3	10.8	22.0	12.0	21.1	15.6
Z. c. 7	25.7	23.7	7.8	23.5	8.6	23.3	9.3	*	*	22.9	10.9	22.8	11.3	22.3	13.3	22.3	13.3	22.0	14.4
Z. b. 4	26.3	24.6	6.5	24.5	6.8	24.4	7.2	24.0	8.7	22.8	13.3	22.6	14.1	22.3	15.2	22.2	15.6	20.9	20.9
Z. b. 3	26.4	25.5	3.3	25.1	4.9	25.1	4.9	24.7	6.4	23.5	7.2	23.2	8.3	22.8	9.9	22.7	10.3	21.2	19.7
Z. c. 8	27.0	25.4	5.9	25.7	4.8	25.7	4.8	*	*	25.4	5.9	24.8	8.2	24.7	8.6	24.3	10.0	24.0	11.1
Average shrinkage in per cent.		10.9		11.5		11.4		11.8		16.2		18.5		19.4		20.5		24.8	

* Not measured in 50 per cent alcohol.



Measurements in Successive Stages of Preparation

Fig. 1 Graph showing the shrinkage induced in pig embryos by the various solutions with which they are treated in the Zenker method of preparing material for sectioning. The extent of the shrinkage is indicated by expressing the embryos' length at various steps in the process as a per cent of their length when fresh. The points on the curve are located from the average measurements of twenty-eight embryos. The embryos when fresh ranged from 9 to 27 mm. in crown-rump length (average length fresh 16.1 mm.). Individual measurements for the same group of embryos are given in table 1.

length (e.g., embryos from 11.9 mm. to 12.2 mm., table 1) shows their shrinkage at various steps in the process to be surprisingly uniform.

The arrangement of the measurements in order of the original length of the embryos brings out another point. The total shrinkage is greater in the younger embryos than in the older. While it would be unsafe to draw any final conclusions from data based on embryos of the limited size range here studied, it seems probable that this difference in shrinkage is due largely to the differences in the organization of the developing muscular and connective tissues. In embryos of about 10 mm. the mesodermal tissues are represented for the most part by loosely aggregated cell masses with abundant interstitial spaces. By the time the embryo has attained a length of 20 to 25 mm. the cells of the developing muscular and connective tissues have become much more compact with a corresponding reduction of the interstitial spaces. Moreover, in the older embryos chondrification has begun in many centers and renders the body of the embryo more rigid as well as more compact. These conditions would seem quite sufficient to account for the fact that fixation and dehydration of very young embryos result in a greater shrinkage than that induced in older embryos.

The extent to which shrinkage varies with the original length of the embryo is shown graphically in figure 2. The curve is not as precisely indicated as it would be by a larger number of cases, but its general course is nevertheless quite apparent. Only measurements of embryos fixed in bichromate fluids were used in plotting this curve since their shrinkage is closely comparable, whereas some of the other fixing fluids show quite different shrinkage curves.

It was thought possible that preliminary coagulation of embryos by treatment in hot water might reduce the amount of shrinkage they suffered. To test this possibility, embryos were immersed for five minutes in water of 65°C. and then put through the routine Zenker process. As will be seen by comparing the curves of figures 1 and 3, this preliminary treatment with hot water proved to be ineffective in reducing the amount of shrinkage.

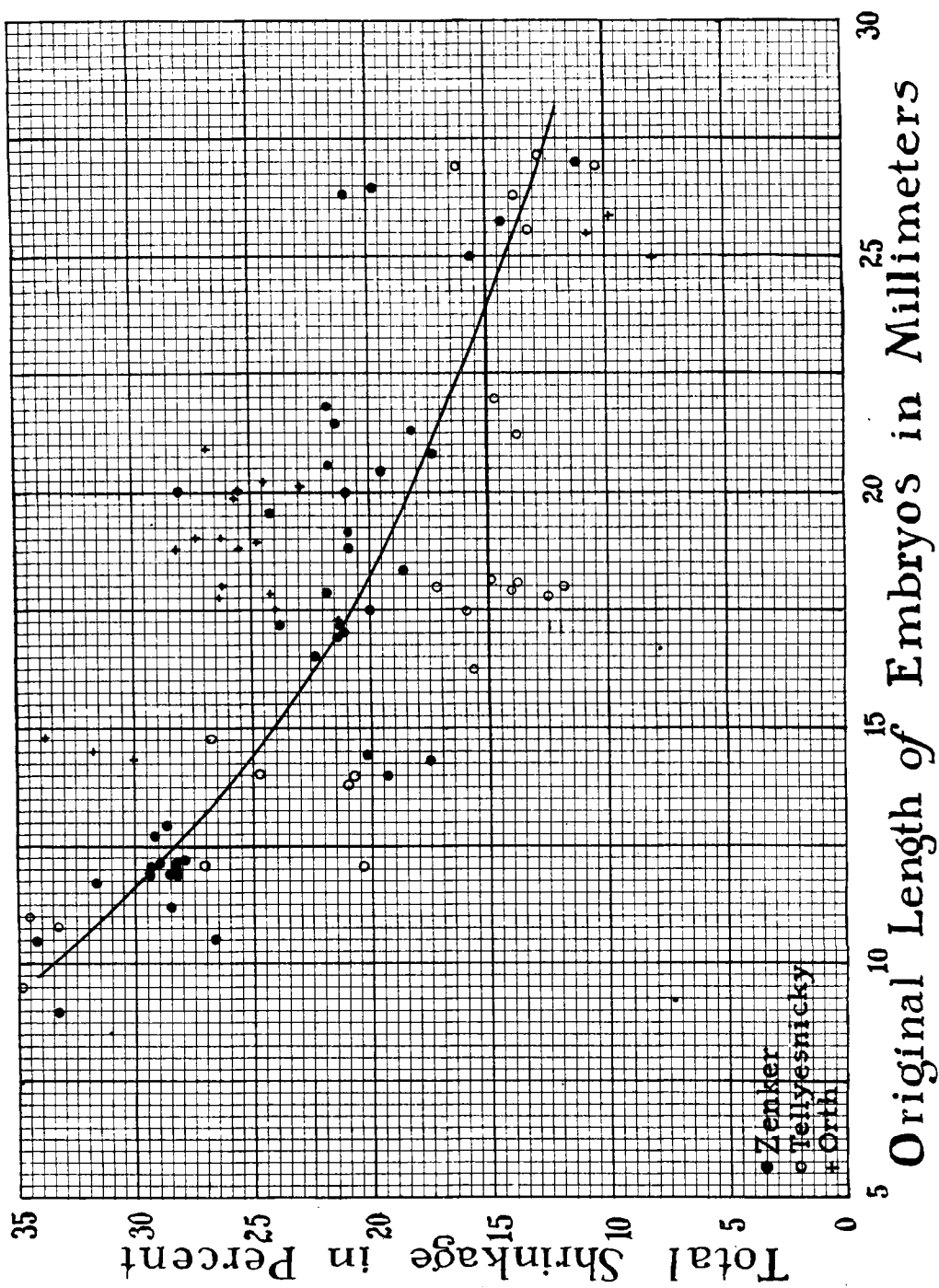
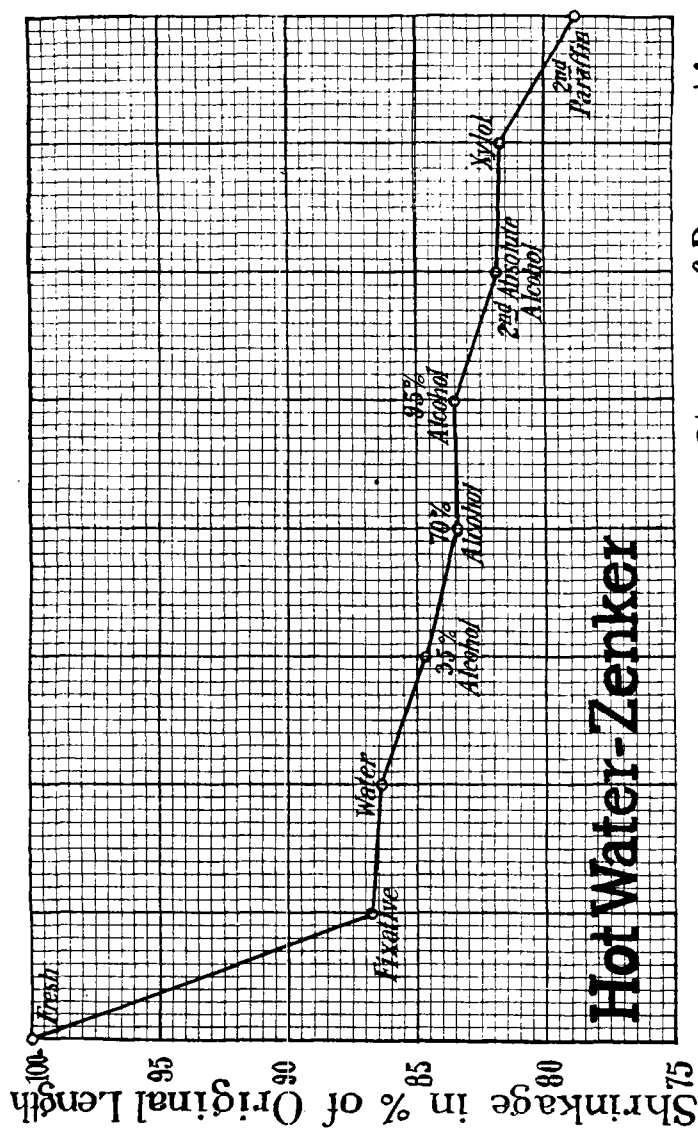


Fig. 2



Measurements in Successive Stages of Preparation

Fig. 3

Fig. 2 Curve showing the extent to which the total shrinkage of embryos during preparation for sectioning varies with the size of the embryo. Each point is located from the measurements of a single embryo.

Fig. 3 Graph showing the shrinkage induced in pig embryos by the various solutions to which they were subjected in the Zenker method modified by treatment for five minutes with water at 65°C. just before immersion in Zenker. The curve is based on the average measurements of fifteen embryos having an average original length of 18.6 mm.

The slightly less extensive shrinkage shown by the embryos treated with hot water before fixation, if significant at all, is attributable rather to the fact that their average length (18.6 mm.) was somewhat greater than the average length (16.1 mm.) of the embryos treated by Zenker alone.

Orth's fluid (formula as given in Lee, '13)

The technique employed with embryos fixed in Orth's fluid and the times the embryos were allowed to remain in the different solutions were essentially the same as that described for Zenker's solution except for the omission of the iodine treatment. The results of the measurements were tabulated as for the embryos fixed in Zenker, but since the measurements were of the same character as those given in table 1 they have not been given in detail. Figure 4 summarizes the results graphically.

The presence of formalin in Orth's fluid might be expected to lessen the shrinkage to a certain extent. Such is apparently the case, for the shrinkage in this fixative is noticeably less extensive than that in Zenker (compare fig. 4 with figs. 1 and 3). When, however, we follow out the curve, we find that the advantage is only temporary. There is a greater shrinkage in dehydration following fixation by Orth than in dehydration following Zenker fixation. The total shrinkage encountered in the two techniques is virtually identical. The transitory nature of the swelling effect of formalin fixation when it is followed by dehydration is shown much more strikingly by the data given below for formalin uncombined with other solutions.

Tellyesnický's fluid (formula as given in Minot, '11)

The technique following fixation in Tellyesnický's fluid was similar to that used for Zenker and Orth material. The results of the measurements are summarized in the curve of figure 5. The slightly smaller amount of shrinkage indicated on the Tellyesnický curve as compared with the other bichromate fixatives is not sufficient to be regarded as significant. Both as regards the total amount of shrinkage induced and the stage of the process

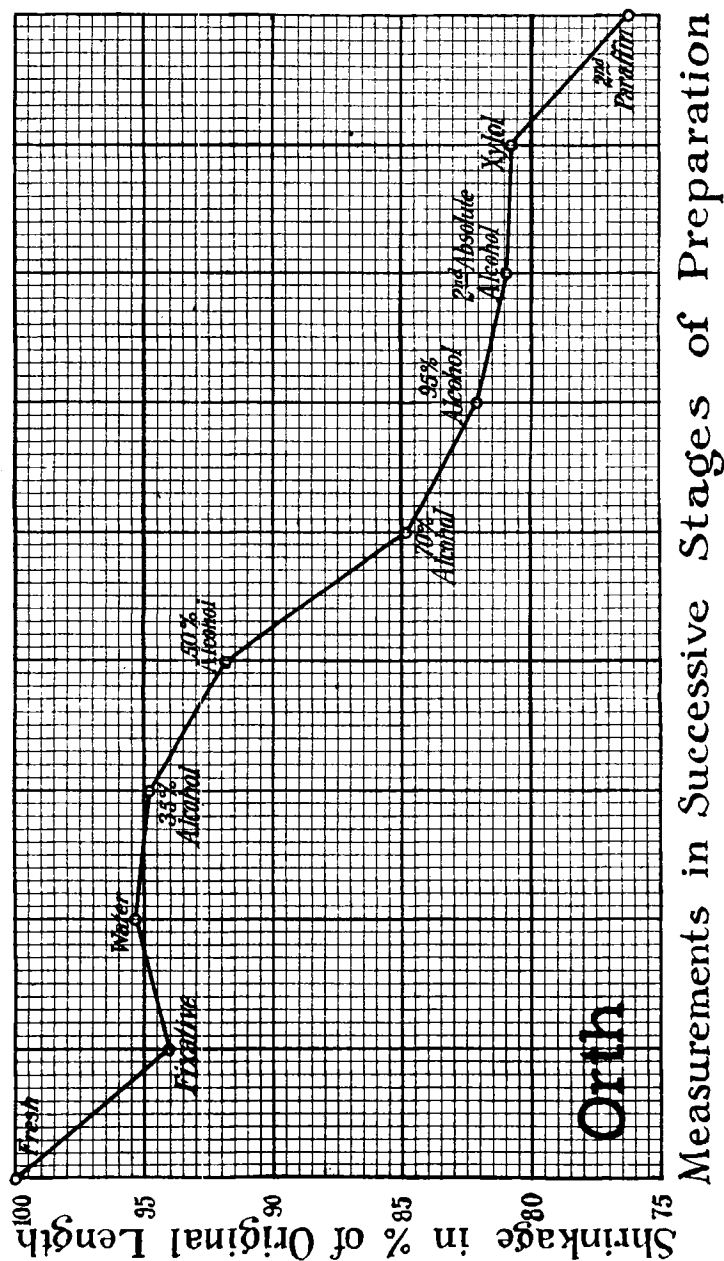
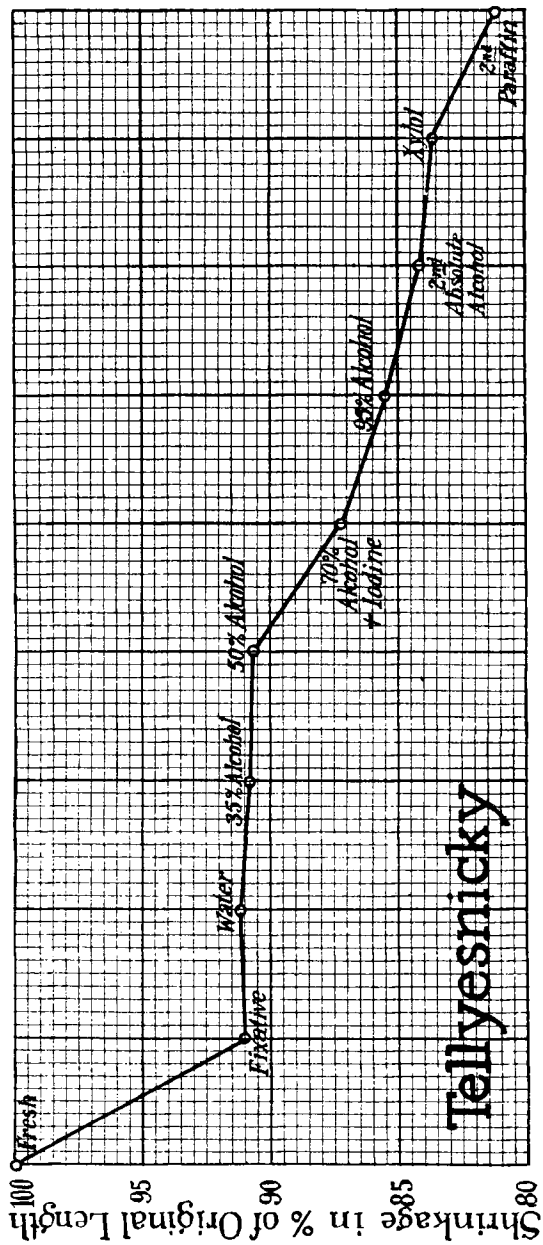


Fig. 4 Graph showing the shrinkage induced in pig embryos following fixation in Orth's fluid. The points on the curve were located from the average measurements of twenty-one embryos averaging 19.7 mm., original length.



Measurements in Successive Stages of Preparation

Fig. 5 Graph showing the shrinkage induced in pig embryos following fixation in Tellyesnick's fluid. The points on the curve were located from the average measurements of twenty-four embryos, averaging 18.3 mm., original length.

at which the shrinkage takes place, Tellyesnický, Orth, and Zenker material behave essentially alike.²

Formalin (10 per cent commercial formalin)

Embryos were fixed in formalin for twenty-four hours, transferred to 70 per cent alcohol overnight, left in 95 per cent alcohol twenty-four hours, in absolute (two changes) four hours, in xylol three hours, in soft paraffin two to three hours, and in hard paraffin two to three hours, according to their size.

The measurements of the embryos at various stages in the process are given in detail in table 2 and summarized in the graph of figure 6. The most striking thing that the measurements for formalin fixed embryos bring out is the fact that the initial swelling induced by the formalin is not only rapidly lost on treatment with alcohols, but gives way to marked shrinkage. In fact, the shrinkage of formalin-fixed material during dehydration is more extreme than the shrinkage encountered in dehydration following the other fixatives we used. While, therefore, formalin fixation causes an initial swelling, the ensuing processes of dehydration and infiltration result in a final shrinkage which is approximately the same as that in the bichromate processes.

Schultz ('19) found for fetuses, as did Hrdlička ('06) and King ('13) for brain material, that the initial swelling effect of formalin fixation tended to become less marked with continued storage in formalin. Although long periods in formalin reduced the initial swelling, it still left their material increased in weight as compared with its fresh condition, producing nothing approaching the shrinkage we found to be caused by dehydration of formalin-fixed embryos.

In regard to dimensions, Schultz found considerable variability, some measurements showing a slight decrease and others

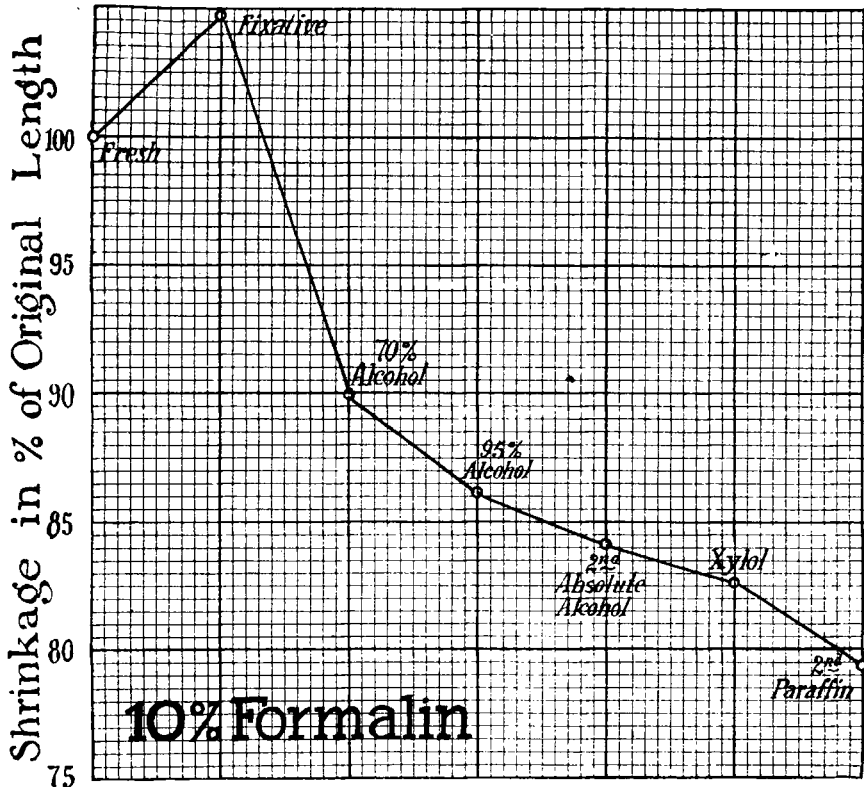
² For adult brain material Donaldson ('94), King ('10), and Plant ('18) found an increase in weight and volume following treatment with bichromate solutions. This is in marked contrast to our findings for embryos. The difference between adult brain tissue with its compactness and high myelin content and young embryos with their loosely organized structures might possibly account for their divergent reaction in fixatives.

TABLE 2
Measurements of pig embryos, showing the amount of shrinkage which occurs in the various solutions used in the formalin (10 per cent) method of preparing material for sectioning

IDENTIFICATION NUMBER OF EMBRYO	C-R. LENGTH IN AMNIOTIC FLUID mm.	10 PER CENT FORMALIN		70 PER CENT ALCOHOL		95 PER CENT ALCOHOL		SECOND ABSOLUTE ALCOHOL		XYLOL		SECOND PARAFFIN	
		C-R. length mm.	Shrink- age per cent	C-R. length mm.	Shrink- age per cent	C-R. length mm.	Shrink- age per cent	C-R. length mm.	Shrink- age per cent	C-R. length mm.	Shrink- age per cent	C-R. length mm.	Shrink- age per cent
F. c. 1	11.8	12.0	+1.7	10.8	8.5	10.1	14.4	9.8	16.9	9.8	16.9	9.0	23.7
F. a. 13	12.4	13.3	+7.0	11.9	3.5	10.6	14.5	10.4	16.1	10.1	18.1	9.9	20.2
F. a. 15	12.7	13.5	+5.5	11.1	12.6	10.9	14.2	10.9	14.2	10.5	17.3	10.0	21.3
F. a. 10	13.0	13.6	+4.6	11.1	14.6	11.0	15.4	10.7	17.7	10.3	20.8	10.0	23.1
F. a. 5	13.0	14.0	+7.7	11.1	14.6	10.8	16.9	10.6	18.5	10.2	21.6	9.9	23.8
F. a. 8	13.8	14.4	+4.4	11.4	17.4	11.0	20.3	11.0	20.3	10.8	21.7	10.5	23.9
F. a. 3	14.6	15.8	+8.2	12.6	13.7	12.0	17.8	12.0	17.8	11.8	19.2	11.3	22.6
F. a. 2	14.7	15.4	+4.8	12.3	16.3	11.9	19.0	11.8	20.1	11.5	21.7	11.1	24.5
F. a. 9	14.7	16.5	+12.2	13.3	9.5	13.0	11.6	12.8	12.9	12.6	14.3	11.8	19.7
F. a. 6	14.9	15.7	+8.1	12.5	16.1	12.2	18.2	12.2	18.2	12.0	19.6	11.3	24.1
F. a. 7	14.9	15.6	+4.7	12.7	14.8	12.1	18.8	12.1	18.8	11.9	20.1	11.5	22.8
F. a. 12	15.8	17.1	+8.2	14.0	11.4	13.5	14.6	13.5	14.6	13.3	15.8	12.8	19.0
F. a. 4	19.0	20.1	+5.8	16.9	11.0	16.0	15.8	15.8	16.8	15.7	17.4	14.9	21.6
F. a. 14	19.1	20.0	+4.8	17.0	11.1	16.3	14.7	16.0	16.3	15.7	17.8	15.1	20.9
F. a. 1	19.5	21.0	+7.7	17.3	11.3	17.0	11.8	16.6	14.9	16.4	16.9	15.5	20.5
F. a. 11	20.0	20.5	+2.5	17.4	13.0	16.5	17.5	16.4	18.0	16.3	18.5	15.3	23.5
F. c. 4	21.8	22.2	+1.8	20.2	7.3	18.2	16.4	18.0	17.3	17.9	17.8	16.5	24.1
F. b. 3*	23.3	24.2	+6.2	22.7	1.8	21.0	10.0	20.2	12.3	19.8	13.9	18.5	18.2
F. c. 2*	24.4	24.9	+2.0	22.7	6.8	20.8	10.8	20.6	11.8	20.0	15.4	17.5	21.1
F. c. 7	24.9	25.1	+0.8	25.1	+0.8	24.8	0.4	24.6	1.2	24.3	2.4	23.1	7.2
F. c. 5	25.1	25.3	+0.8	24.8	1.2	24.6	2.0	24.1	4.0	24.0	4.4	23.0	8.4
F. c. 6	25.8	25.9	+0.4	25.1	2.7	24.8	3.9	24.7	4.3	24.4	5.5	23.4	9.3
Average shrinkage.....		Swelling of 4.8 per cent		Shrinkage of 10 per cent		13.8 per cent		15.9 per cent		16.4 per cent		20.6 per cent	

* All the embryos in this series were measured both for crown-rump length and belly thickness. For the most part the per cent of shrinkage for the two methods of measurement corresponded very closely. In the case of the embryos indicated in the table with an asterisk these two measurements showed unusual divergence. For this reason the shrinkage figures given for them are the mean of the crown-rump and belly shrinkage. All other values are for crown-rump measurements.

a slight increase after storage in formalin. The great difference in the age of the embryos he worked on, as compared with those we dealt with, makes it inadvisable to attempt a direct comparison of our results.



Measurements in Successive Stages of Preparation

Fig. 6 Graph showing the shrinkage induced in pig embryos following fixation in 10 per cent formalin. The points on the curve were located from the average measurements of twenty-two embryos, averaging 17.7 mm., original length. Individual measurements for the same group of embryos are given in table 2.

Formol-alcohol

There are various mixtures of formalin and alcohol in use as fixatives. The formula we used was:

Formalin, (commercial).....	100 cc.
Alcohol, 95 per cent.....	450 cc.
Acetic acid (glacial)	20 cc.
Distilled water.....	430 cc.

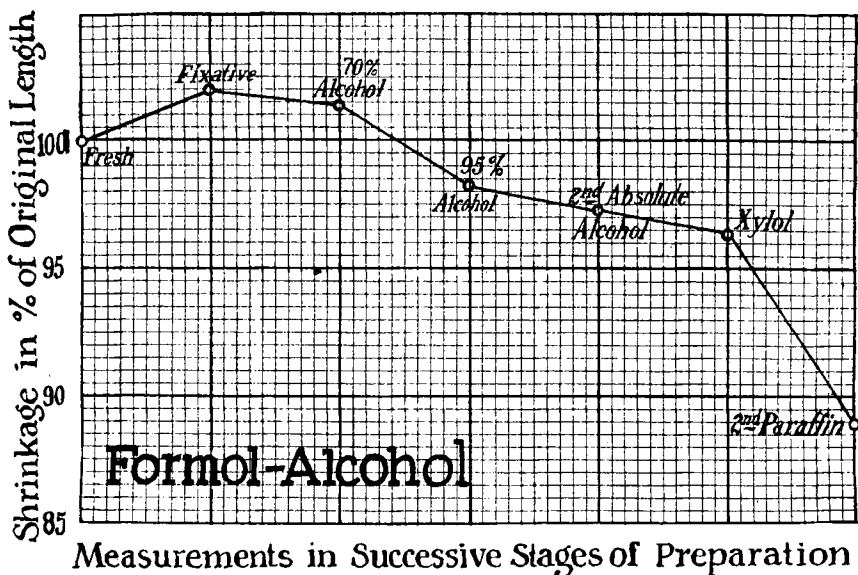


Fig. 7 Graph showing the shrinkage induced in pig embryos following fixation in formol-alcohol. The points on the curve were located from the average measurements of twenty-six embryos, averaging 19.8 mm., original length.

The technique used following formol-alcohol was similar to that given above for 10 per cent formalin. The results of the measurements are summarized in the curve of figure 7.

In embryos of the age range we worked with there was a slight initial swelling produced. This swelling was by no means as marked as that in 10 per cent formalin and could probably be altogether done away with by using more alcohol in the fixative.

The curve of figure 7 would seem to indicate that preservation of gross material with virtually no distortion from its fresh

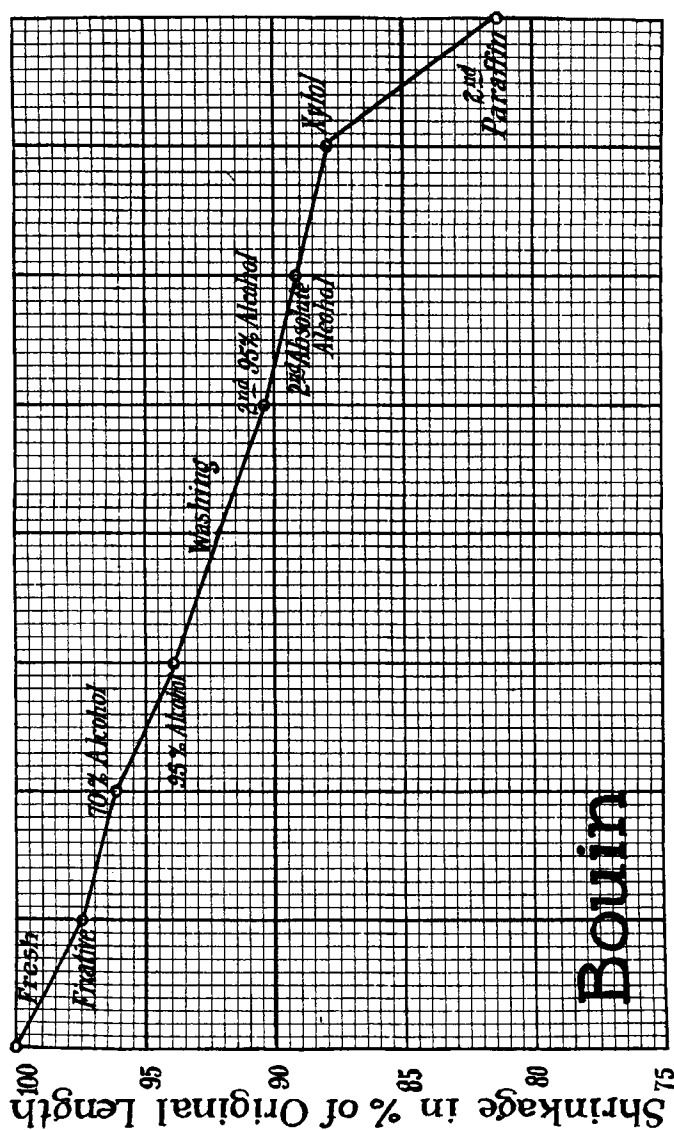
measurements could be secured by storing material in alcohol of about 80 per cent. However, further work on this point is necessary, because, as the results of Schultz referred to above clearly show, long-continued storage in a preservative may result in unexpected changes in dimensions.

As a fixative for material to be used for histological purposes, little can be claimed for formol-alcohol. While the distorting effect of the fixative itself could undoubtedly be nullified for embryological material of a given stage as was done by Parker and Floyd ('95) for nervous tissues, there would still remain the very considerable shrinkage in dehydration and infiltration. It is true that the total shrinkage in the formol-alcohol technique, as indicated by the graph of figure 7, is somewhat less than in the bichromate fixatives, but the slight advantage on that score is more than counterbalanced by its marked inferiority as a preservative of cytological detail.

Bouin's fluid (formula as in Lee, '13)

Embryos were fixed in Bouin fifteen to eighteen hours, transferred to 70 per cent alcohol for four hours, and then to 95 per cent alcohol for twenty-four hours for hardening. After hardening they were run back through the alcohols and washed in running water overnight, and then run up to absolute, being left four hours in each of the ascending alcohols. The removal of the picric acid by this method instead of by 70 per cent alcohol was done as a matter of economy of alcohol. Except where a large quantity of material is being handled, it is without advantage. With the preliminary hardening in 95 per cent alcohol it appeared to have no deleterious effect on the fixation.

The measurements for Bouin material are summarized in figure 8. The shrinkage in the fixative is very slight (average 2.5 per cent) and in the remainder of the process is gradual and not excessive. The total shrinkage of embryos in the Bouin technique is considerably less than that encountered in the more usually employed bichromate fixatives. The preservation of cytological detail by Bouin is in no way inferior to that secured



Measurements in Successive Stages of Preparation

Fig. 8 Graph showing the shrinkage induced in pig embryos following fixation in Bouin's fluid. The points on the curve were located from the average measurements of twenty-three embryos, averaging 19.8 mm., original length.

by Zenker, Orth, or Tellyesnicky, and greatly superior to that secured by formalin or formol-alcohol. As to favorability for staining, Bouin material, if thoroughly washed, leaves little to be desired.

SUMMARY AND CONCLUSIONS

To ascertain the amount of shrinkage which is induced by preserving embryological material and preparing it for sectioning, series of measurements were made on pig embryos covering each stage of some of the common techniques.

The fixatives used were: Zenker, Orth, Tellyesnicky, 10 per cent formalin, formol-alcohol, and Bouin. The crown-rump length of the embryos was measured first in the amniotic fluid, and thereafter following each solution with which they were treated. The average shrinkage was computed in per cent of the original length of the embryo. From these averages graphs were plotted to show the shrinkage encountered at each step of each of the techniques used.

The shrinkage in the bichromate fixatives, Zenker, Orth, and Tellyesnicky, was the greatest encountered, the decrease in crown-rump length ranging from about 30 per cent in 10-mm. embryos to about 20 per cent in 20- to 25-mm. embryos. The more extensive shrinkage in the younger embryos is probably attributable to the less compact condition of their mesoderm. The greatest part of the shrinkage in these techniques came in the fixatives themselves.

Fixation in 10 per cent formalin resulted in an average increase in crown-rump length of about 5 per cent, which was, however, followed by rapid shrinkage during dehydration and infiltration. The total shrinkage of formalin material by the time it was embedded in paraffin was scarcely less than that for material fixed in the bichromate solutions. Histologically, the formalin-fixed material was distinctly inferior.

Formol-alcohol fixation resulted in a slight initial swelling, which was followed by marked shrinkage in dehydration and infiltration. The total shrinkage was somewhat less than that encountered in the bichromate fixatives, but the histological condition of the material was not as good.

Embryos treated with Bouin's fluid showed very slight shrinkage in the fixative. A gradual shrinkage continued, however, during dehydration and infiltration. The total shrinkage in the process was somewhat less than that following the bichromate solutions, and the histological condition of the material and its stainability were fully as satisfactory.

Comparison of the graphs for the several techniques shows:

1. There is a general tendency for the shrinkage in dehydration to be greater in the processes where the shrinkage in the fixative is less. This practically nullifies, as far as material for sectioning is concerned, the supposed advantages of a mixture such as formol-alcohol which is compounded to avoid shrinkage in the fixing fluid itself.

2. There is an abrupt increase in shrinkage during paraffin infiltration which is about the same for all the techniques used. This shrinkage encountered in molten paraffin would in all probability not occur in celloidin embedding, and would be largely reduced by the use of the celloidin-paraffin or chloroform-paraffin method of infiltration.

The crown-rump length of subsequently prepared sagittal sections showed no change from the crown-rump length of the embryo from which they were cut, as measured after paraffin infiltration.

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