TABLE II

AUXIN CONTENT IN TOMATO SEEDLINGS. THE AUXIN CONCENTRATION IS DENOTED IN TERMS OF INDOLE ACETIC ACID EQUIVALENTS, AND THE FIGURES ARE GAMMAS PER KG FRESH MATERIAL

Time of extraction	Conc. of auxin	Time of extraction	Conc. of auxin
1939–			
4–10	3.98	8–27	1.76
	*1.06	9–18	9.55
	*1.82	25	1.07
15	1.87	10– 3	0.60
22	2.27	7	1.09
29	0.97	14	0.96
5– 4	1.00	24	0.76
11	0.91	11– 4	1.30
21	1.07	18	1.95
25	0.78	21	1.91
30	1.30	28	0.5
6– 5	1.31	12– 8	1.54
7	1.12	15	0.74
15	1.03	1940–	
7–13	0.68	1– 5	0.93
15	1.36	16	1.47
19	1.21	27	1.9
21	1.20	2–10	1.33
24	1.50	20	1.24
27	1.50	27	1.76
31	0.96	3– 9	2.39
8– 4	1.17	23	2.50
9	2.29	4– 8	2.50
14	†1.62	5– 6	3.66
17	1.58		

* Refluxed 4¼ and 3 hours, respectively.
† Material frozen and ground finely.

from a lot of plants, was there in an active form at the time the plants were collected.

DuBuy's[3] method of extracting the frozen and ground plant material with cold water has not been found to be satisfactory. Avery's[4] recently published method of extracting with alcohol, while satisfactory with dried corn, does not work with fresh plants. Thimann[5] used chloroform as a solvent, and in an experiment with *Malvaviscus* sp. it has been compared with ether. The total auxin obtained in 14 extractions was nearly twice as great with ether as with chloroform.

Table III shows the importance of proper dilution.

TABLE III

INFLUENCE OF DILUTION UPON THE APPARENT AMOUNT OF AUXIN OBTAINED. THE AUXIN CONCENTRATION IS DENOTED IN TERMS OF INDOLE ACETIC ACID EQUIVALENTS, AND THE FIGURES ARE GAMMAS PER KG OF FRESH MATERIAL. THE AMOUNT OF MATERIAL WAS THE SAME IN ALL DETERMINATIONS

Amount of agar in cc added to the extract				
.6	.7	1.0	1.5	2.0
2.00		3.64	5.06	
3.20		3.84	7.65	
	3.38	4.10	6.32	6.28
2.49		3.72	6.07	5.94

Of the solvents used ether seems to be the best, but it does not extract all the auxin except after many extractions. Care must be taken to have proper dilutions. This has previously been discussed by Went and Thimann with respect to indole acetic acid but not previously stressed with extracted material.

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A SIMPLE METHOD OF OBTAINING PURE CULTURES OF EMBRYONIC HEART MUSCLE

A BRIEF statement concerning the cultivation of embryonic chick heart muscle *in vitro* may serve a twofold purpose, adding to the data available on the tolerance of tissue cells from warm-blooded animals to subnormal temperatures[1] and suggesting a possible method of obtaining pure cultures of embryonic heart muscle.

Fragments of 8-day embryonic chick heart muscle were explanted to a medium consisting of 1 drop of fowl plasma (dil. 2:1 with Tyrode solution) plus 2 drops of freshly prepared 8-day embryonic juice (dil. 1:2 with Tyrode solution). One culture of a limited series remained in the incubator continuously for a period of 18 days without renewal of the nutrient medium. The temperature during incubation varied between 33°–35° C.

Microscopic examination of the living culture on the eighteenth day of cultivation revealed a network of refractile cells which were contracting vigorously. The nuclei of the cells appeared unusually clear with particularly prominent nucleoli. Connective tissue cells had entirely disappeared from the culture.

Microscopic examination of the fixed and stained preparation (Champy-Kull process followed by Heidenhain's iron alum haematoxylin method) showed that histodifferentiation had not taken place. No striated myofibrils were present in the network of cells. There was an accumulation of uniformly fine fat droplets in a relatively small number of cells, while a still smaller number contained large vacuoles. The nuclei of the vacuolated cells showed evidence of degenerative changes. No mitotic figures were present.

The fact that the muscle cells remained functional while all types of connective tissue cells degenerated suggests a simple method of obtaining pure cultures of embryonic chick heart muscle. The more usual method of subdividing cultures at the time of transplantation is worthless in the case of heart muscle,

[3] H. G. DuBuy, *Jour. Agr. Res.*, 56: 155–158. 1938.
[4] G. S. Avery, *Am. Jour. Bot.*, 26: 679–682. 1939.
[1] A. Fischer, *Arch. f. exper. Zellforsch.*, Bd. 2, S. 303–305, 1925–26; L. Buccianti, *ibid.*, Bd. 11, S. 397–423, 1931; E. F. Stilwell, *ibid.*, Bd. 21, S. 447–476, 1938; D. C. Hetherington and J. C. Craig, *Jour. of Cell. and Comp. Phys.*, 14: 197–203, 1939.
[5] K. V. Thimann, *Jour. Gen. Physiol.*, 18: 23–34. 1934.

since disturbance of tensional stresses set up within the culture leads to the eventual degeneration of the muscle cells.

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EVALUATION OF ISOBUTYL METHACRYLATE POLYMER AS A MOUNTING MEDIUM

APPARENTLY on the strength of the recent article by O'Brien and Hance[1] regarding the use of isobutyl methacrylate polymer as a mounting medium for covering histological sections, a scientific instrument and supply company has stocked the material and has recommended it in the house organ of the company. Inasmuch as the article of O'Brien and Hance is misleading, I should like to refute their claims to the end that many microscopists might be spared disappointment.

A year and a half ago I acquired some isobutyl methacrylate monomer. Shortly thereafter I found that the polymerized material is not satisfactory as a mounting medium either with or without a cover glass. It does not adhere as well to glass as do the other resins used. This is, admittedly, of slight import if the slides are handled with ordinary care. Certain stains are faded rapidly by it. It softens and decomposes at relatively low temperatures. Hence slides covered with it can not be used in a carbon arc projector unless precautions which restrict the usefulness of the projector are taken to keep the point at which light is directed from getting hot.

The fourth, and major, objection to isobutyl methacrylate polymer is the fact that its refractive index, 1.477, is much lower than that of any of the tissue elements which remain in an ordinary animal tissue section prepared for histological work. For example, the refractive index of unstained striated muscle after being treated as in ordinary histological procedure is 1.537 ± 0.003. The refractive index of stained tissue is still higher than that of unstained tissue. The relatively great difference between the refractive indices of tissues (mainly denatured proteins) and isobutyl methacrylate polymer accounts for the observation of O'Brien and Hance that their preparations were "somewhat more brilliant when viewed under the microscope than are specimens mounted in Clarite." The brilliancy is simply the general effect of the Becke line,[2] which is undesirable, at least for most microscopical work including photomicrography. Inasmuch as the concentration of the stain in the tissue is low, the dispersion curve, refractive index (n) plotted against wave-length (λ), will be nearly regular. The ideal medium for stained sections would be one for which the dispersion curve, n plotted against λ, is the same as the dispersion curve for the stained tissue. The stained tissue elements then would be opaque only because of their absorption of light of certain wave-lengths. This ideal medium can not be realized because the different tissue elements of the section do not have the same dispersion curves, and indeed the same elements of a given section may vary slightly among themselves. For practical purposes, a mounting medium should be used which has a refractive index for the D line of the spectrum as close as possible to that of most of the stained tissue elements.

From the point of view of refractive index alone, Clarite X is the best mounting medium on the market for critical work with animal tissues. Its refractive index is 1.567. This is very slightly lower than that of most stained elements, but is appreciably closer than any other medium available. Also, if there are any unstained or faintly stained elements in the section they are less apt to be missed with Clarite X than with Clarite, gum damar or Canada Balsam inasmuch as the refractive indices of the latter three are much closer to the refractive index of unstained tissue elements than is Clarite X. Clarite has an index of 1.544. Sections mounted in it can be distinguished readily from those in Clarite X upon examination under the microscope. The refractive index of gum damar is 1.542. That of Canada Balsam is still lower, 1.535. The refractive index of isobutyl methacrylate polymer, 1.477, is quite low in comparison to the above, and makes the material of little use as a mounting medium except possibly in special cases where a very low refractive index is desired.

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[2] C. M. Goss, *Arch. f. exper. Zellforsch.*, Bd. 12, S. 233–248, 1932; E. F. Stilwell, *ibid.*, Bd. 21, S. 447–476, 1938.

[1] H. C. O'Brien and R. T. Hance, SCIENCE, 91: 412, 1940.

[2] A short discussion of the Becke line can be found in A. N. Winchell, "Elements of Optical Mineralogy," Part I, 5th ed., John Wiley and Sons, Inc., New York, 1937.

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