reasonable approximation to the original curve. A still better fit might have been obtained from means differing from the given mean by tenths of a degree, instead of by the half-degree intervals fixed upon to simplify computations.

Equal standard deviations. Assuming the two presumed components to have equal standard deviations, instead of assuming values for their means, led Charlier (*1*) to a cubic equation involving the difference between the variances of the given distribution and the assumed components:

$$z^3 + \tfrac{1}{2}(\nu_4 - 3\sigma^4)z + \tfrac{1}{2}\nu_3^2 = 0,$$

where $z = \sigma_1^2 - \sigma^2$. The discriminant of this cubic,

$$C^2 = (\sigma^{12}/216)\ (13.5\alpha_3^4 + E^3),$$

where $\alpha_3 = \nu_3/\sigma^3$ is the skewness and $E = (\nu_4/\sigma^4) - 3$ the excess, almost always is positive, indicating only one real root:

$$z = 0.4082\ \sigma^2\ (\sqrt[3]{-3.6742\alpha_3^2 + \gamma}\ -\ \sqrt[3]{3.6742\alpha_3^2 + \gamma}),$$

where $\gamma = \sqrt{13.5\alpha_3^4 + E^3}$.

Except for almost symmetrical and very flat-topped distributions, γ is positive, so that z will be negative, and

$$\sigma_1^2 < \sigma^2.$$

But if $-z > \sigma^2$, then σ_1^2 is negative, and there is no actual solution, indicating the assumption of equal standard deviations to be unwarranted; for Jacksonville July temperatures, variances assumed to be equal are -5.07.

If the assumption is justified, and σ_1 is real, the means are:

$$M_1 = M - m_1 = M - (\nu_3/6) - \sqrt{(\tfrac{1}{4}\nu_3)^2 - z}$$

$$M_2 = M + m_2 = M - (\nu_3/6) + \sqrt{(\tfrac{1}{4}\nu_3)^2 - z}.$$

The areas N_1 and N_2 of the two components are found as before.

An asymmetrical curve which is the sum of two normal curves "affords a good fit both to distributions which possess two distinct modes, and to skewed distributions with one mode" (*8*). That components have not been found generally for such distributions may be due to ignorance of Charlier's facile methods; this ignorance, in turn, may stem from Pearson's insistence (*6*), replying to Edgeworth's criticism (*4*), that his "process is not so laborious that it need be discarded for rough methods of approximation based upon dropping the fundamental nonic and guessing suitable solutions."

Of Charlier's methods, the first, that of assumed means, is far simpler than the second, which involves the fourth moment and a cubic equation. However, Charlier concentrated on the second, the "abridged method for dissecting frequency curves," because the cubic equation involved is actually one step in the general solution, "hence it is no loss of time to begin with this approximate method."

Charlier declared that the assumption that the standard deviations of the two components are equal "is of a more general character" than the assumed knowledge of the means of the two components. "Especially in biology it is a fairly probable supposition that two types found together in nature often possess *nearly* equal standard deviations," but "this abridged method is applicable only when there are a priori reasons for the assumption that the two components have nearly equal standard deviations." In many cases no such reasons exist, and then it is safer to assume certain values for the means of the components, especially when approximate means may be determined by inspection. In the example given, the approximate values of the means were obvious from the graph, and trial of a few pairs of values yielded one which gives a good fit, with markedly different standard deviations; assumption of equal deviations gave no solution.

Even closer agreement with the original distribution can be obtained if values may be assumed for the maximum ordinates as well as the means of the two components. In effect, this short cut replaces the standard deviation and skewness of the original distribution by a subjective evaluation which may be more effective for some distributions, but is not of as general applicability in finding two normal components.

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Combination of Tissues from Different Species in Flask Cultures[1]

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Combining tissues from different species apparently has been performed only infrequently in tissue culture. Roffo (*5*) grew together chicken and rat tissues, both normal and neoplastic, without evidence of antagonism. In studies of the mode of transmission of the excitation involved in cardiac muscle contraction, several investigators (for reference see Leone [*3*]) combined embryonic heart fragments of the chick with similar fragments from other avian and mammalian species. The establishment of synchrony of beat was reported, and no mention was made of any incompatibility reactions.

Harris (*2*), in an attempt to determine whether direct incompatibility exists between tissues of different mammalian species in culture, paired heart, spleen, and kidney fragments from newborn mice and rats in roller tubes. Harris found that ". . . rat and mouse tissue cells are physiologically compatible *in vitro.*" In a related study, though not involving tissues from different species, Medawar (*4*) recently made combined fluid cultures of skin from two adult rabbits, between which skin grafts had failed to take, and found no evidence of incom-

[1] With the technical assistance of Clara Lee and Edward J. Soban.

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patibility. The present report confirms these earlier observations, in that no overt incompatibility has been found in combined cultures of tissues from mouse and rat, mouse and guinea pig, and mouse and chicken, even when the mouse tissues were derived from animals previously immunized against the opposite species.

TABLE 1

SUMMARY OF MOUSE-RAT TISSUE COMBINATIONS

Mouse (strain C)	Rat (strain Marshall or Hairless)	Duration of experiment (days)	Total number cultures
Kidney, 16-day embryo	Kidney, embryo, near term	6	2
" adult	" adult	6	2
Spleen, 16-day embryo	Spleen, embryo, near term	6–11	5
" adult	Kidney, embryo near term	6	2
" "	" adult	6	2
" "	Spleen, "	6	1
			14

All cultures were carried in modified Carrel D-3.5 flasks. Aseptically prepared tissue fragments, 1–2 mm in diam, were planted several millimeters apart in a clot of 0.6 ml chicken plasma and 0.8 ml of a nutrient fluid consisting of 2 parts horse serum,[3] 2 parts Tyrode's solution, and 1 part chick embryo juice. After the clot formed, 1.0 ml of the nutrient fluid was added to each flask. Three times weekly the nutrient was drawn off, the cultures were washed with 2.0 ml of Tyrode's solution, and fresh nutrient added. In some cases (see Tables 2 and 3), the chick embryo juice was omitted from the nutrient in order to slow the rate of growth.

TABLE 2

SUMMARY OF MOUSE-GUINEA PIG TISSUE COMBINATIONS

Mouse (strain C)	Guinea pig (family 13)*	Duration of experiment (days)	Total number cultures
Kidney, 16-day embryo	Kidney, embryo, near term	4	3
Spleen, 16-day embryo	Kidney, embryo, near term	4	1
Spleen, 16-day embryo	Spleen, embryo, near term	4	2
Spleen, adult	Spleen, adult	5	3
" "	Kidney, young, 7–9 days	11	5
" " †	Kidney, young, 7–9 days	11	6
" " ‡	Kidney, young, 7–9 days	11	6§
Lymph node adult	Kidney, young, 7–9 days	11	11§
Lymph node adult†	Kidney, young, 7–9 days	11	11§
Lymph node adult‡	Kidney, young, 7–9 days	11	5§
			53

* Inbred line developed by Dr. Sewall Wright and maintained by Dr. Walter Heston at this institute.

† Donor mouse immunized with guinea pig serum.

‡ Donor mouse immunized with guinea pig kidney.

§ Chick embryo juice omitted from nutrient fluid in one half of cultures.

Tables 1–3 list the various tissue combinations studied. Strains and ages of donors are given in the tables. Omitted are the controls which were run in each experiment and which consisted of cultures of paired tissue fragments from one species—either mouse, rat, guinea pig, or chicken.

Early contact of individual cells, as well as the later stages of growth, was observed microscopically. At intervals, representative cultures were photographed and, in some cases, fixed and stained, using a modification of the technique of Earle (*1*).

TABLE 3

SUMMARY OF MOUSE-CHICKEN TISSUE COMBINATIONS

Mouse (strain C)	Chicken (stock)	Duration of experiment (days)	Total number cultures
Kidney, 16-day embryo	Heart, young, 7–9 days	21	9*
Kidney, young, 7 days	Heart, young, 7–9 days	21	9
Spleen, 16-day embryo	Spleen, young, 7–9 days	4	2
Spleen, young, 7 days	Heart, young, 7–9 days	21	9*
Spleen, adult	Kidney, young, 7–9 days	4	3
" "	Heart, young, 1 day	8	9
" " †	Heart, young, 1 day	8	9
			50

* Chick embryo juice omitted from nutrient fluid in one half of cultures.

† Donor mouse immunized with chicken serum.

To determine whether immunization of one species against antigens from another affects the reactions of their tissues when combined in culture, groups of young mice (5–7 weeks old) were injected with either guinea pig serum (diluted 1: 7 with saline), guinea pig kidney (brei diluted 1: 10), or chicken serum (diluted 1: 3). The immunization course consisted of intraperitoneal injections twice weekly for 3 weeks. The total amount of diluted antigen given was 4.5 ml. One week after the final injection the mice were bled, along with controls, and the desired tissue taken for culture. Pooled serum samples were tested for antibody content by the "ring" precipitin test. Antibody titers of 1: 64 in the anti-guinea pig sera, and 1: 640 in the anti-chicken serum.

[3] Kindly supplied by Dr. Wilton R. Earle.

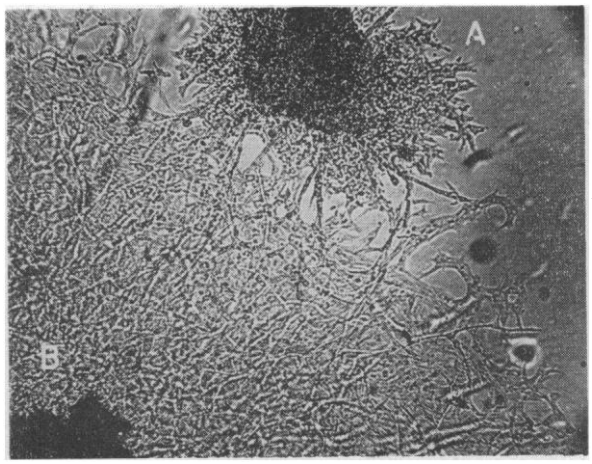

FIG. 1. Four-day combined culture of (A) spleen of mouse immunized with chicken serum, and (B) 1-day-old chick heart. Note that outgrowth zones have joined and that there is no evidence of antagonism. Magnified 90 ×.

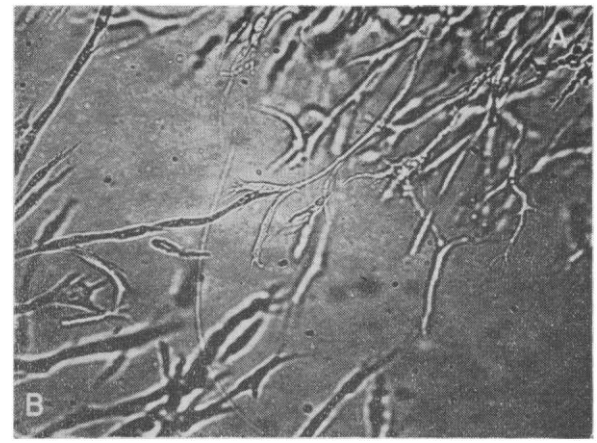

FIG. 2. Four-day combined culture of (A) lymph node of mouse immunized with guinea pig serum, and (B) 3-day-old guinea pig kidney. Intimate contact of normal cells from the two species can be observed. Magnified 280 ×.

During the first few days of incubation, extensive cellular migration occurred from all explants. In cultures of tissues from different species, cells at the margins of the outgrowth zones came into intimate contact with each other (Figs. 1 and 2) as in cultures of tissues from the same species. In both types of culture, the cells in the contact zone, as elsewhere, appeared normal. The cells were observed to come together in the contact zone and, as migration continued, the margins of the outgrowth zones were joined without any sharp demarcation. There was no evidence of: 1) abnormal cellular accumulation or reaction along the line of junction between the two explants, 2) altered rate or direction of growth in the contact area, or 3) specific attraction or antagonism between cells of the two species. Once the growth zones joined, continued cultivation with or without embryo juice, up to a maximum of 21 days, produced no further change, apart from progressive growth of the cultures.

The growth patterns described were identical in the case of combinations involving explants from previously immunized animals. Tissues of immunized mice, as compared with those of nonimmunized mice, showed no difference in reaction to tissue fragments from the species that furnished the antigens.

The results show that tissues of two different species, as widely diverse as mouse and chicken, may be grown simultaneously in flask culture without apparent antagonism. This is true even when the donor mouse has been previously immunized against antigens of the species furnishing the other tissue of the paired combination, and when the mouse tissue is one which *in vivo* presumably is intimately involved in antibody formation, i.e., spleen and lymph node. At present, the chief significance of these findings is seen in the possibility that such combined cultures may provide a method for investigations requiring maintenance of healthy tissues of two species in close physiological relations.

Further investigation is necessary before these results may be interpreted in relation to problems of antibody production *in vitro* and of incompatibility in transplantation *in vivo*. Interpretation at present is hindered for the following reasons: First, no attempt was made to provide optimum conditions for antibody production, or its detection in the culture fluids. Second, the media used contained components heterologous for both species, thereby complicating immunological interpretations. Finally, in the present state of knowledge of cellular physiology in culture, it is not safe to transfer directly to the organism conclusions based upon observations of cellular behavior *in vitro*.

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Transphosphorylation by Alkaline Phosphatase in the Absence of Nucleotides[1]

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Axelrod (*1*), using citrus fruit phosphatase, observed the transfer of phosphate from nitrophenyl phosphate to methanol in the absence of nucleotides. The transferred phosphate did not pass through the inorganic stage. Recently, we observed with alkaline intestinal phosphatase a strong acceleration of the rate of synthesis of glycerophosphate from inorganic phosphate and glycerol, in the

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