IN VITRO AGGREGATION OF MIXED EMBRYONIC KIDNEY AND NERVE CELLS

Influence on Macromolecular Synthesis

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ABSTRACT

The possible role of nerve on growth of embryonic parenchymal organs such as kidney was explored by measuring macromolecular synthesis (DNA, RNA, and protein and three enzymes) in aggregates of mixed suspensions of cells from dissociated chick embryo kidney and nerve tissue. One and one-half to threefold increments in net synthesis of the three different types of macromolecules were observed in the mixed aggregates of kidney and nerve cells as compared with those of single organs or mixtures of kidney with nonneural cells. The addition of nerve-growth factor (NGF) did not significantly affect the results. Increased incorporation of label was paralleled by increases in chemically measured DNA and protein, suggesting an increase in growth in the mixed kidney-nerve aggregates compared with those of single tissues. Measurements of survival rate did not indicate increased cell stability in the mixed aggregates. The activities of three enzymes, acid phosphatase, alkaline phosphatase, and lactic dehydrogenase, were also enhanced two to four times in cultures of kidney plus nerve cells. Morphologic studies indicated a high degree of reorganization of tubular structures within the reaggregates of kidney cells alone or in those mixed with nerve. In addition, radioautographs of thymidine-3H-labeled cells in the aggregates showed a high level of DNA synthesis in the reformed tubular cells. Electron micrographs revealed the presence of large numbers of nerve fibers containing microtubules in the mixed cell aggregates. The data suggest a significant role for nerve in the growth processes of embryonic parenchymal organs.

In contemplating the possible types of cell-to-cell interactions which might play an important role in certain phases of organogenesis in the embryo, we were struck with the potential importance of a nerve supply. The "trophic" influence of nerve on the development and maintenance of muscle is a well-known example (Guth, 1968). Perhaps the most dramatic instance of morphogenetic dependence upon nerve is the requirement for a critical level of axonal volume for normal limb regeneration in amphibians, so amply documented by Singer and colleagues. Apparently, the type of nerve is not critical; motor, sensory, or autonomic will do (see recent reviews by Singer, 1965, 1968). Recently, Dresden (1969), who studied the role of nerve in newt limb regeneration, observed a marked diminution in RNA, DNA, and protein synthesis in the "paddle" stage regenerates within 24 hr after denervation. This effect was not noted in the proximal stump or in the contralateral nonamputated but denervated limb. In addition, he observed that the effect could be found in
in vitro behavior of the regenerates; those from denervated limbs showed considerable reduction in macromolecular synthesis as compared with innervated controls in organ culture. Lebowitz and Singer (1970) subsequently confirmed and extended these observations with regard to protein synthesis in vivo and also found that crude peripheral nerve homogenates infused into denervated regenerating limbs largely restored protein synthesizing activity. These last observations strongly favor a "trophic" factor rather than synaptic-mediated stimulation.

There are numerous other instances of the influence of nerve on various phases of growth, development, and maintenance of a variety of tissues. The development of the mesonephric blastema stimulated by close contact with spinal ganglia in the absence of Wolffian duct, has been reported by Gruenwald (1942). Grobstein (1955) observed that the dorsal half of the spinal cord would induce secretory tubules in explanted 11 day mouse embryo mesonephric mesenchyme through a Millipore filter, and the ventral half of the spinal cord promotes chondrogenesis in cultured somites (Holtzer, 1961). Maintenance, function, and regeneration of taste buds are closely dependent upon the integrity of the gustatory nerves (Zelenas, 1964; Oakley and Benjamin, 1966; Guth, 1969); the metabolic activity of brown adipose tissue is strongly influenced by sympathetic activity (Sidman and Fawcett, 1954), and Overton (1950) observed mitotic stimulation of amphibian epidermis by implanted grafts of central nervous tissue.

Current literature lends support to the argument that nerve stimulation enhances synthetic activity in several fully differentiated tissues. Shimazu and Amakawa (1968) reported that stimulation of the splanchnic nerve caused an increase in the activities of two glycolgenolytic enzymes (glycogen phosphorylase and glucose-6-phosphatase) in rabbit liver. The activity of another liver enzyme, tyrosine aminotransferase, is sensitive to peripheral autonomic neurotransmitter substances; the cholinergic agent, carbachol, initiates a twofold rise in rat liver enzyme activity which is abolished when the cholinergic blocking agent, atropine, is injected simultaneously (Black, 1970). Also, the possible neurohumoral agent, serotonin, has been shown to increase the incorporation of radioactive thymidine into guinea pig skin in vitro (Mann, 1967) and to increase the growth of fibroblasts in culture (Boucek and Alvarez, 1970).

In order to explore the possibility that association with nerve might play a significant role in development and growth of parenchymal organs, we have examined the influence of dissociated sensory and sympathetic ganglia cells on macromolecular synthesis of reaggregated renal cells of the chick embryo in vitro. In experiments reported here suspensions of dissociated ganglia cells were mixed with equal numbers of parenchymal organ cells, principally kidney, and the synthesizing abilities of the mixed reaggregates were compared with those of reaggregated cells from each tissue alone. The incorporation of labeled precursors into DNA, RNA, and protein as well as the activity of three enzymes were used as indices of the effect of interaction of neurons with parenchymal cells on growth of reaggregates in vitro. Also, the morphology of the reaggregates was examined. Nerve-growth factor (NGF)1 was used in duplicate sets of cultures from each experiment with the hope of increasing the neurogenic influence.

**Methods**

Dorsal root ganglia, sympathetic ganglia, spinal cord, neural retina, and liver were obtained from 8-day chick embryos. 3-day embryos were the source of limb bud, skin, and heart. Metanephros was removed from 11-day embryo chicks. Kidney, liver, and other nonnervous tissues were dissociated into single cells by exposure to 2% trypsin 1-300, 1% pancreatin 3X (both, Sigma Chemical Co., St. Louis. Mo.) in Ca++- and Mg++-free Moscona’s solution for 30 min at 37°C (Auerbach and Grobstein, 1958). Neural retina was dissociated after a 10 min incubation in equal volumes of the trypsin-pancreatin solution and 0.1% Versene. The ganglia and sympathetic chain combined were incubated in the trypsin-pancreatin-versene solution for 1/2 hr. Trypsin was removed and replaced with 50% horse serum and 50% Tyrode’s medium. The tissue was then transferred to a test tube containing 10 ml of horse serum-Tyrode’s solution and 0.1% Versene. The ganglia and sympathetic chain combined were incubated in the trypsin-pancreatin-versene solution for ½ hr. Trypsin was removed and replaced with 50% horse serum and 50% Tyrode’s medium. The tissue was then transferred to a test tube containing 10 ml of horse serum-Tyrode’s solution and, after the pieces had settled to the bottom of the tube, 9 ml of the liquid was withdrawn. Dissociation into single cells was accomplished by agitating the tissue in the remaining 1 ml on the Vortex Jr. Mixer for approximately 15 sec. The cells were counted in a hemocytometer, and a total of 5 x 10⁵ cells in 5 ml of 199 medium containing 5% horse serum were used.

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1 Abbreviations used in this paper: NGF, nerve-growth factor; NADH, nicotinamide adenine dinucleotide; TCA, trichloroacetic acid.
serum, 5% embryo extract, 100 units/ml penicillin plus streptomycin, 50 units/ml mycostatin were placed in a 25 ml Erlenmeyer flask. The flasks were gassed with 5% CO2-95% air and incubated at 37°C on a rotary shaker set for 70-80 rpm (Mosecona, 1961). When two different types of tissue were grown in the same flask, 2.5 × 10^6 cells of each type were used, thereby maintaining the same final concentration of 5 × 10^6 cells per flask.

10 units of NGF obtained from the Wellcome Research Laboratories, Beckenham, England, or 2 µg of NGF kindly supplied by Dr. Milton Levy were added to one of duplicate flasks in each experiment each day in culture.

Radioactive precursors (1 µCi/ml thymidine-3H, 0.36 Ci/m mole; 0.1 µCi/ml leucine-14C, 180 mCi/m mole; and 1 µCi/ml uridine-3H, 2 Ci/m mole) were added to the flask either every day or 24 hr before harvest, as specified for each type of experiment.

The cells were collected by centrifugation and washed several times with isotonic saline. When leucine-14C or thymidine-3H had been used, the aggregates were dissolved in 1 ml of 1 N NaOH at 37°C for 1/2 hr. Protein and DNA were precipitated by the addition of 1 ml of 50% trichloroacetic acid (TCA). The precipitate was washed with 10% TCA and taken up in 1 ml of 0.2 N NaOH. The tissue dissolved yielding a uniform suspension, and a 0.2 ml sample was removed for counting in Bray’s solution containing cabosil in an automated liquid scintillation counter with a counting efficiency of 85% for 14C. When uridine-3H was used to label the cells, the tissue was precipitated with TCA directly after washing, omitting the first exposure to NaOH. The remainder of the procedure was identical to that described above. Protein was determined by the Lowry procedure (Lowry et al., 1951) and DNA by the diphenylamine reaction (Burton, 1956).

For the enzyme analyses, the contents of two to four flasks each containing aggregates of 5 × 10^6 cells (initial inoculum) were combined after 4 days in culture and sedimented in the cold in an International centrifuge. The pellet was washed three times with saline and resuspended in a small volume of either water or the appropriate buffer. The suspension was sonicated and spun at 10,000 rpm in the Sorvall RC2-B centrifuge for 30 min, and the supernatant was used for the assays. Acid phosphatase was measured according to the method of Brandenberger and Hanson (1953) and Hofstee (1954). Increase in absorbancy at 300 mp due to the liberation of salicylic acid from 8-carboxyphenylphosphate was followed in the Zeiss spectrophotometer. 1 unit is defined as 1 µmole of 8-carboxyphenylphosphate hydrolyzed/min at 25°C. Alkaline phosphatase activity was measured according to the method of Garen and Levinthal (1960). The rate of release of p-nitrophenol from p-nitrophenylphosphate was followed by observing the increase in absorbancy at 410 mp. A unit is defined as activity liberating 1 µmole of p-nitrophenol/min at 25°C. Lactic dehydrogenase activity was determined by following the decrease in absorbancy at 340 mp due to the oxidation of nicotinamide adenine dinucleotide (NADH) (Kornberg, 1955). 1 unit is defined as that NADH/min at 25°C. For the three enzymes the results are expressed as units per culture flask and units per milligram protein.

Morphologic examination of aggregates of mixed and homogeneous cell populations was performed on glutaraldehyde-OsO4-fixed specimens after 48 and 72 hr in culture. These were embedded in Epon, sectioned, and examined by phase-contrast and electron microscopy. In addition, radioautographs were prepared from Epon-embedded sections of 2-day mixed and single tissue aggregates after incubation for another 24 hr with thymidine-3H.

RESULTS

Incorporation of Labeled Precursors

In the majority of the experiments the tissues were kept in culture for a total of 4 days, and radioactive precursors were added on day 3, 24 hr before harvesting the aggregates. The results are summarized in Fig. 1. The data are reported as the ratio of observed incorporation in mixed aggregates to the incorporation in control cultures of kidney and nerve alone, to enable us to compare experiments with each other in a simple manner. However, the raw data for one experiment of each type are presented in Table I. Each measurement is the number of counts per minute per flask, each of which was inoculated with the same number of cells. In all instances the actual radioactivity counted ranged between 10^6 and 10^7 cpm. The mean and range of complete replicate experiments are indicated on the bar graphs in Fig. 1. The observed incorporation for the mixture of cells averaged 80% greater than 1/4 the sum of an equal number of nerve and kidney cells aggregated separately. NGF did not significantly affect the results, as shown in Fig. 1, and the experiments with NGF may be considered as an additional set of replicate experiments. Note that incorporation of label in mixtures of kidney and liver showed no increment above that of either tissue alone.

The results with uridine-3H were more pronounced. The middle group of bars (Fig. 1)
represents the average of two experiments. Mixed aggregates of kidney plus ganglia incorporated about 2.5 times more radioactive label than aggregates of each tissue alone. Again, with NGF added to the media, the incorporation of label was not significantly greater than that in cultures of mixed aggregates which did not receive the factor. Mixtures of kidney and liver cells showed no greater incorporation than half the sum of that for each tissue alone.

Incorporation of leucine-14C into protein was also enhanced in mixed aggregates although the per cent of stimulation was less than that observed with thymidine or uridine; an average of 1.4 times greater labeling than in controls, and when NGF was added the stimulation of incorporation was not significantly higher. Liver plus kidney mixtures, again, remained at the control level. From Table I it is clear that, concomitantly with the enhanced incorporation of radioactive precursors by mixed aggregates containing nerve, there is an almost parallel increase in the chemically measured amount of protein and DNA. The amount of RNA per flask was not determined. The increment in protein in the mixed aggregate cultures containing nerve was about 70% above the controls of each tissue cultured alone; DNA was about 60% higher. Mixed cultures of kidney plus liver did not exhibit the enhanced incorporation of radioactive precursors, nor did the protein and DNA increase.

Because, in the mixed aggregates, the radioactivity per flask and the total DNA or protein per flask rose concomitantly, the use of specific activities to express the data would have obscured the obvious acceleration of growth that occurred when nerve was mixed with kidney.

Table II summarizes the results of several other control experiments in which kidney was mixed with a number of other tissues. With the exception of kidney plus spinal cord and neutral retina, the ratios all hover close to 1, indicating that the two exceptions, both neuronal, behave like ganglia cells.

The time course of enhancement of macromolecular synthesis was examined by adding isotopically labeled precursors each day during the culture periods of 24, 48, and 72 hr. As with the single application of label, mixed aggregates of kidney and nerve synthesized 1.5-2 times more DNA, RNA, and protein than controls of each tissue alone after 72 hr of growth. As Table III indicates, however, this enhancement was not apparent until the third day in culture. Aggregates of kidney plus liver cells showed no increment in incorporation of any of the three labels during the entire time period.

**Specific Enzyme Activities**

Three enzymes were examined as indices of the effect of nerve-kidney cell interactions on specific
Table I
Macromolecular Synthesis in Mixed and Separate Cell Aggregates

| Label          | K     | N     | K+N   | R    |
|----------------|-------|-------|-------|------|
| Leucine-^{14}C|       |       |       |      |
| cpm/flask \(\times 10^{-3}\) | 1.99  | 0.83  | 2.44  | 1.73 |
| \(\mu g\) protein/flask | 738   | 560   | 1113  | 1.71 |
| cpm/\(\mu g\) protein | 2.8   | 1.5   | 2.2   | 1.03 |
| Thymidine-^{3}H|       |       |       |      |
| cpm/flask \(\times 10^{-4}\) | 10.62 | 6.38  | 11.44 | 1.34 |
| \(\mu g\) DNA/flask | 18.2  | 12.4  | 24.8  | 1.62 |
| cpm/\(\mu g\) DNA | 5878  | 5050  | 4660  | 0.85 |
| Uridine-^{3}H |       |       |       |      |
| cpm/flask \(\times 10^{-4}\) | 6.83  | 1.80  | 10.74 | 2.49 |

Flasks were labeled after 3 days in culture. The aggregates were harvested 24 hr later on day 4 as described in Methods. R is the ratio of values for mixed cell aggregates to values for controls of each cell type grown separately. The control incorporation is calculated by adding one-half of the actual counts per minute observed in separate cultures initially inoculated with \(5 \times 10^6\) kidney cells and \(5 \times 10^6\) nerve cells (or liver cells). The ratios of experimental to control for the lines giving the protein and DNA content per flask are calculated by dividing the micrograms of protein or DNA in the mixed culture flask by the sums of one-half the micrograms of protein or DNA in flasks of each tissue cultured separately. K, kidney; L, liver; N, nerve.

Table II
DNA, RNA, and Protein Synthesis in a Variety of Mixed Cell Aggregates Comparing Those Which Include Nerve with Those Which Do Not

| Tissue type               | Experiment No. | R* DNA | Experiment No. | R RNA | Experiment No. | R Protein |
|---------------------------|----------------|--------|----------------|-------|----------------|-----------|
| Kidney and liver          | 10             | 1.1    | 4              | 1.02  | 6              | 1.02      |
| Kidney and limb bud       | 2              | 0.7    |                |       |                |           |
| Kidney and heart          | 2              | 1.14   |                |       |                |           |
| Kidney and skin           | 2              | 0.88   |                |       |                |           |
| Kidney and neural retina  | 6              | 1.60   | 12             | 1.31  |                |           |
| Kidney and spinal cord    | 4              | 1.80   |                |       |                |           |

* The ratio R is defined in Fig. 1 and Table I, and the values represent the mean of the data for the replicate experiments of a type. Half of the replicate experiments had NGF added but are included since the effect was not significant.
macromolecules. Acid phosphatase, alkaline phosphatase, and lactic dehydrogenase were chosen because they are active enough in the cultured tissue for quantitation in the small amount of protein per culture flask. The results, summarized in Table IV, show that mixed aggregates of kidney plus nerve have 1.4- to 4-fold greater enzyme activities than either kidney or nerve aggregates separately. These results are in agreement with the isotope incorporation data presented earlier with respect to increments of activity per flask; however, there is also a concomitant increase in specific activities, i.e., units per microgram protein not seen in the labeling of DNA or protein.

The ratios of enzyme activities for aggregates of kidney and liver remained at 1 or below in all cases.

### Table III

**Kinetics of Incorporation of Radioactive Precursors into Cultures of Mixed Aggregates**

| Day | R DNA | R RNA | R Protein |
|-----|-------|-------|-----------|
| Kidney and nerve | | | |
| 1   | 0.84  | 1.15  | 1.06      |
| 2   | 1.11  | 1.16  | 1.09      |
| 3   | 1.83  | 1.74  | 1.35      |
| Kidney and liver | | | |
| 1   | 1.20  | 0.99  | 1.10      |
| 2   | 0.79  | 0.84  | 1.12      |
| 3   | 1.01  | 1.00  | 1.01      |

The numbers in this table are the ratios explained in the legends of Fig. 1 and Table I.

### Table IV

**Activities of Three Enzymes in Culture**

| Enzyme                  | Kidney and nerve | Kidney and liver |
|-------------------------|------------------|------------------|
|                         | K                | N                | K + N             | R      |
| Alkaline phosphatase    | 7.40 X 10^4      | 4.65             | 15.40             | 2.54   |
| units/flask             | 1.78 X 10^3      | 2.07             | 2.72              | 1.41   |
| Acid phosphatase        | 10.95 X 10^4     | 4.02             | 30.40             | 4.05   |
| units/flask             | 1.77 X 10^3      | 1.62             | 5.26              | 3.10   |
| Lactic dehydrogenase    | 7.35 X 10^2      | 0.90             | 11.85             | 2.87   |
| units/flask             | 11.90 X 10^2     | 3.60             | 20.60             | 2.68   |

| Enzyme                  | K                | L                | K + L             | R      |
|-------------------------|------------------|------------------|------------------|--------|
| Alkaline phosphatase    | 7.40 X 10^4      | 6.50             | 7.49             | 1.06   |
| units/flask             | 1.78 X 10^3      | 1.90             | 1.95             | 1.06   |
| Acid phosphatase        | 10.95 X 10^4     | 15.55            | 10.75            | 0.81   |
| units/flask             | 1.77 X 10^3      | 2.80             | 1.93             | 0.84   |
| Lactic dehydrogenase    | 7.35 X 10^4      | 6.00             | 2.65             | 0.67   |
| units/flask             | 11.90 X 10^2     | 1.10             | 4.80             | 0.73   |

Units are defined under Methods. R, the ratio, is defined in the legends for Fig. 1 and Table I.
**Table V**

| No. of flasks | Cell aggregate | Incubation | Cells medium $\times 10^{-3}$ (Range) | Survival % |
|---------------|----------------|------------|--------------------------------------|------------|
| 2             | Kidney         | 0.5        | 2.78-3.41                            | 73         |
| 1             | Ganglia        | 0.5        | 19.6                                 | 91         |
| 1             | Kidney + ganglia | 0.5       | 15.7                                 | 92         |
| 5             | Kidney         | 72         | 0.75-0.87                            | 28         |
| 2             | Ganglia        | 72         | 8.25-8.75                            | 36         |
| 5             | Kidney + ganglia | 72        | 4.13-5.57                            | 35         |

**Cell Survival**

As a quantitative measure of the relative amount of cell death and dissolution in the mixed cultures as compared with those of the separate organs, a group of eggs were injected with tritiated thymidine (50 $\mu$Ci per egg) on 3 consecutive days, after which kidneys and ganglia were harvested, separated into single cell suspensions, and reaggregated as described. One flask of each group was harvested after 30 min and also 72 hr in culture. The cells were separated from the medium of each flask by centrifugation and each group of cells was washed once in the centrifuge in fresh medium. The cell pellets were dissolved in 0.1 N NaOH after homogenization, and samples of the initial medium, wash medium, and dissolved cells were assayed for radioactivity. From the ratios of radioactivity in the cells as compared to the medium (Table V), there would appear to be a similar survival rate in the mixed cultures as compared with the calculated values for kidney alone plus ganglia alone, i.e., 35% compared with 32%. The results of two such experiments were essentially the same.

**Morphology**

Histologic examination of the aggregates from dissociated metanephros alone and in combination with nerve cells examined 48 and 72 hr after the reassociation began showed the expected organization into characteristic renal tubules surrounded by numerous, randomly distributed cells, the identity of which could not be determined. In some instances, a well-ordered layer of columnar epithelial cells more or less completely surrounded the reaggregates. Nothing resembling glomeruli was observed, nor was it possible to distinguish nerve elements within the aggregates at the histologic level in the absence of specific staining (Fig. 2). In radioautographs of reaggregates exposed to thymidine-3H, the highest concentrations of grains were found over the nuclei of the organized tubular cells, although there were significant amounts of nuclear uptake of label in cells of the less well-organized areas of the aggregate (Fig. 3). Seemingly equivalent extent and localization of labeling occurred in aggregates of kidney cells alone.

Electron micrographs of thin sections through these aggregates indicated the reappearance of most of the structural organization seen in the intact embryonic kidney. Well-developed basement membrane had reformed close to the outer surface of the tubular cells, outside of which layers of collagen fibrils and less well-organized, presumably mesenchymal and nerve cells could be seen. Facing the lumen of the reorganized tubules, typical microvilli were observed. Large numbers of neuronal fibers containing large bundles of microtubules and filaments were seen coursing between the mesenchyme and renal tubule cells as seen in Fig. 4. Detailed description of the fine structure of reaggregated kidney and of the neuron-kidney cell relationships will be reported at a later date.

Although the mixed cultures containing nerve, especially those with NGF in the medium, seemed healthier in that their aggregates were larger and more numerous, the degree of histologic organization was not demonstrably greater than in aggregates of kidney cells alone.

**Discussion**

The data presented here suggest that nerve, at least in embryonic tissues, has a stimulatory effect on growth and proliferation of parenchymal
FIGURE 2  Phase-contrast micrograph of a thin section of 4 day reaggregated kidney plus ganglia cells in the presence of NGF. Magnification, 440.
Figure 3  Radioautograph of tritium labeled 4 day reaggregate of kidney and ganglia cells in presence of NGF. Magnification, 440.
FIGURE 4  Electron micrograph of 4 day reaggregated kidney and ganglia cells exposed to NGF. This figure illustrates bundles of axonal microtubules in association with parenchymal cells. Between the cells collagen fibrils can be seen. Magnification, 40,000.
organs. This phenomenon may indicate a more
generalized "trophic" role for nerve in organo-
genesis, analogous to its role in amphibian limb
regeneration.

The actual measurements during tissue recon-
struction from a cell suspension of dissociated 11
day chick embryonic kidney show that nerve sig-
ificantly enhances DNA, RNA, and protein
synthesis as well as the activities of several enzymes.
Preliminary experiments suggest that the phe-
omenon is a general one and not confined to
kidney tissue exclusively; reaggregated liver plus
nerve also exhibits increased DNA and protein
synthesis.

Enhanced macromolecular synthesis and ele-
vated enzyme activities observed in the nerve-
kidney aggregates cannot be attributed to a simple
interaction between two different cell types since
cells from other embryonic tissues tested were
unable to stimulate macromolecular synthesis in
reconstituted kidney cultures. The effect seemed
not to be limited to sympathetic or sensory cells
since nerve tissue of two other types, spinal cord
and neural retina, also appeared to enhance
growth in mixed cultures.

The nature of the stimulation achieved is sug-
gested by the observation that, although there
was a significant increase in incorporation of
label into the three macromolecular species, there
is a concomitant increase in the amount of chemi-
cally measurable DNA and protein. These data
suggest an increase in cell number, i.e., in
growth rate. However, there does appear to be
significant increments in specific activities of three
enzymes which may indicate increases in certain
intracellular activities as well. These effects cannot
be ascribed to enhanced cell survival in view of the
observations reported in Table V.

Quantitation of cell death by the amount of
solubilized, labeled DNA after 3 days of incuba-
tion seemed to show rather high values; however,
there are no published reports of quantitative
measurement of this kind in this type of aggregate.
Staining methods were not appropriate since the
aggregates could not be readily disaggregated for
cell counts. Histologic and radioautographic ex-
amination, as shown, revealed very few pyecotic
nuclei, a high mitotic rate, and an active thymi-
dine-3H incorporation. It is likely that the con-
siderable cell death at 72 hr compared to 30 min
of incubation represented loss of unaggregated cells
and perhaps over-large aggregates.

Unfortunately, it was not possible in these
experiments to determine which cell type, tubular,
mesenchymal, or neuronal, was principally
affected by the mixed nature of the cultures.
Radioautographs utilizing tritiated thymidine
indicate the most concentrated labeling to be in
the organized tubules; however, this observation
applied to the mixed nerve-kidney preparations
as well as to kidney cell reaggregates alone.
Quantitation here has not yet been attempted.

The degree of structural reordering from a
suspension of individual differentiated kidney cells
was manifested by the appearance on the electron
microscope level of both the intra- and extra-
cellular structures.

There is no clear evidence in the literature to
suggest that the nerve supply is important to the
function of a postembryonic kidney, since the
organ is primarily innervated by perivascular
autonomic nerves which can be stripped away
without significantly interfering with renal func-
tion. However, this fact sheds no light on the
possible involvement in the nervous system in
embryonic growth and development of the organ.

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