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THE USE OF WHOLE ANIMALS VERSUS ISOLATED ORGANS OR CELL CULTURE IN RESEARCH

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As government regulations for animal care increase in number and complexity, and as animal-rights activists continue to push for decreased use of animals for research, more laboratories have turned to tissue and cell culture for biological research. Initial costs of large animals and escalating maintenance costs have driven some researchers from the use of large animal models. Both models—whole animals for chronic experiments and the use of isolated tissue—can give answers to physiological questions. Cost is certainly a factor that must be considered in the present atmosphere in which funding is so difficult to obtain. However, any information gained in isolated tissue must eventually be assessed in the whole animal, where many factors interact to control physiological mechanisms.

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Many reasons have been advanced for the use of isolated tissue rather than whole animals for the study of physiological mechanisms. The cost of using whole animals has risen considerably as the Federal government continues to issue regulations for the care and maintenance of the animals. These regulations have made the cost of whole animal care for chronic experiments increase at a rapid rate. In a study of data collected from 1974 to 1977 (Fitzgerald, 1983), it was reported that the *per diem* cost for care of dogs in a general animal facility increased by 55.17% during that time period. One researcher estimated that the increased cost of animals will come to $98,870 in the three and one half years remaining of his grant. This figure does not include the inceased cost of care associated with new regulations (Glantz, 1989). Antivivisection lobbying has made it increasingly difficult to obtain dogs and cats for such experiments, with sure death for the animals seemingly preferable to their use in scientific research. The difficulty in obtaining animals has raised the cost of each cat or dog so high that most laboratories cannot afford to use them. Therefore many researchers have turned to isolated-organ or cell culture.

What are some of the advantages of such research? One of course is cost. Many experiments can be run on one animal by dividing the tissue into different experimental groups or by doing multiple tests on different sections of the same tissue. Another distinct benefit is the ability to test one substance and to be able to say that any response noted is most likely due to that substance. In this way many chemicals or drugs can be tested to see if the isolated organ, tissue, or cell is capable of responding to that substance. In the whole animal it is not easy to determine the specific effect of any one drug because of the possibility that known or unknown endogenous substances are interacting with the exogenous material being tested. Another advantage is time. In my area of study, using whole animals takes considerable time: it usually takes one whole day to do one experiment. Organ or tissue culture experiments are usually done with multiple samples at one time, and for a shorter duration than with the whole animals.

If there are so many advantages, why not do all research using culture techniques? One must keep

in mind that in the whole animal there are many factors interacting to bring about any response noted in physiological studies. For instance, it is known that pepsin secretion from the chief cells of the stomach is influenced by vagal (Magee, 1982) and sympathetic (Kondo and Magee, 1977; Magee, 1976) innervation, by gastrin from the pyloric area of the stomach (Dutt and Magee, 1972), by secretin (Nakijima et al., 1969; Stening et al., 1969b), and cholecystokinin (Magee and Nakamura, 1966; Stening et al., 1969a; Sjodin, 1972) from the duodenum, by the presence of food (Schofield, 1957; Yagi et al., 1984; Watanabe et al., 1986) and by stretch of the stomach wall (Harper et al., 1959; Magee et al.,1985). Many neurotransmitters are known to be co-localized with acetylcholine or noradrenalin in the nerve endings that innervate the gastric mucosa (Andrews, 1986; Polak and Bloom, 1986). Many functions have been suggested for these transmitters which include Vasoactive Intestinal Polypeptide (VIP), substance P, dopamine, ATP, and GABA. New peptides are being isolated from the gastric and intestinal mucosa on a regular basis, and physiological functions have been suggested for some of them (Konturek et al., 1977; Vagne et al., 1981; Adrian et al., 1985; Berger and Raufman, 1985; Kontourek et al., 1987). How all of these interact in the whole animal is still unclear, or is it known if they act physiologically at all.

Some substances that produce a response in the whole animal do not produce a response on isolated cells or in other denervated tissue. In the innervated stomach of the dog, the hormone gastrin increases pepsin secretion (Kondo and Magee, 1977). When pepsin secretion is determined in the denervated Heidenhain pouch, gastrin has no effect on secretion (Kondo and Magee, 1977). In isolated cell cultures, gastrin again has no effect (Sanders et al., 1983). If only cell culture studies were used to determine the effects of gastrin on pepsin secretion, it would be stated that it has no effect. In truth, it does increase pepsin secretion but only when the tissue is innervated. In our laboratory (Murphy, 1989) we found a difference in the pepsin secretion response to feeding in innervated and denervated gastric pouches stimulated by the duodenal hormone, secretin (Fig.1). Secretin stimulated pepsin secretion from both pouches, unlike gastrin which acted only on the innervated pouch. After feeding, pepsin secretion was inhibited in the denervated pouch but increased in the innervated pouch. Obviously secretin must have some interaction with nervous innervation resulting in the different results observed after feeding.

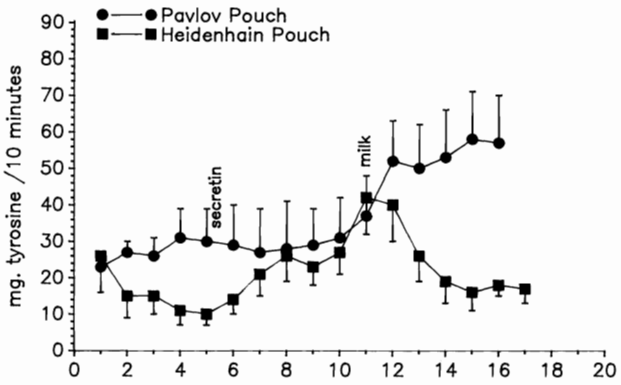

Figure 1. A comparison of pepsin secretion in the innervated Pavlov pouch and the vagally denervated Heidenhain pouch. Points are means ± S.E.M. H.P.: n = 6. P.P.: n = 7.

In cell culture studies both gastrin and cholecystokinin (CCK) increase acid secretion, but only CCK increases pepsin (Sanders et al., 1983). In the whole animal these are competitive antagonists, CCK inhibiting gastrin stimulated acid (Gillespie and Grossman, 1964). Pepsin secretion is stimulated by gastrin in the whole animal (Kondo and Magee, 1977). While varying results have been found with CCK, in most cases it is inhibitory to pepsin secretion (Gillespie and Grossman, 1964).

In the whole animal there are many mechanisms that act to regulate each physiological mechanism, some stimulatory and some inhibitory. In cell culture it is possible to study one of these at a time, but there is no way to study all of the possible interactions that can occur in physiological circumstances. One important aspect of pepsin secretion, the effect of food, cannot be studied in isolated cells. Food in itself is a word that includes any number of substances, and in innumerable combinations. It is difficult to compare literature on the effects of feeding because every laboratory uses different foods in different combinations (Schofield, 1957; Guldvog and Getz, 1981). The different foods contain not only varying amounts of carbohydrates, fat, and proteins, but also different types of these compounds. Various breakdown products of nutrients can stimulate secretion (Yagi et al., 1984) and individual amino acids and fats have been tested as well (Watanabe et al.,1986). But no meal consists of isolated fats, sugars, or amino acids. In cell cultures, cells can be exposed to these substances, but again this can determine if the cell can react. It is not able to determine the reaction when all con-

stituents of food are present, as is the case when a meal is eaten. This is the information that is needed in trying to explain the physiological control of pepsin secretion.

Each type of research has an important place in determining a physiological mechanism. Cell culture studies can determine if a substance can act at the cellular level. If it is found that a substance such as gastrin does not act at this level, then it may be acting at another site in the whole animal or through an intermediary substance. If a pepsin stimulant or inhibitory substance is found to act on the cells themselves, whole-animal studies must be done to determine if they do indeed have a role in the integration of the mechanism under consideration. Any substance studied in isolated systems must eventually be tested in the whole animal. Lesser cost, time commitments, etc. of tissue culture studies, cannot eliminate the need to eventually determine the results in whole animals. The information we are seeking is not the effect on isolated cells, but how the mechanism is regulated under the myriad of interactions that occur in the whole, living animal.

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