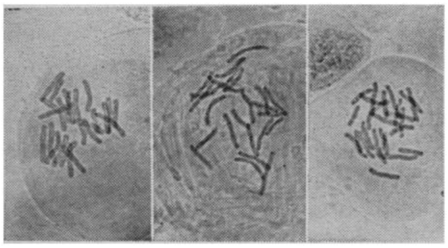

Fig. 1. Metaphase plates from three typical cells of *Picea glauca* tumor grown in vitro.

during these periods. Later work by Kessel (*5*), however, based on much larger numbers, failed to substantiate any preferred time of day or any date earlier than about 21 days.

When properly squashed, individual chromosomes were easily distinguishable (Fig. 1), and only occasionally was there difficulty in counting. To insure that the colchicine was not altering the normal cell structure, several mitotic figures were obtained from untreated material. In some of the controls, and occasionally in the colchicine-treated cultures, obscurities made the use of a camera lucida desirable. In these cases the fiugres were drawn and the chromosomes were then counted from the drawings.

The chromosome numbers are shown in Fig. 2. Of the 300 cells counted, 270 (90 percent) had a (diploid) chromosome number of 24. This is the number given by Sax and Sax for *Picea glauca, P. pungens,* and *P. abies* (*3*). There were seven tetraploid nuclei (48 chromosomes) which is probably not an abnormally high number of polyploids (2.3 percent). The remaining 23 were distributed among 9 aneuploid numbers and may represent counting errors.

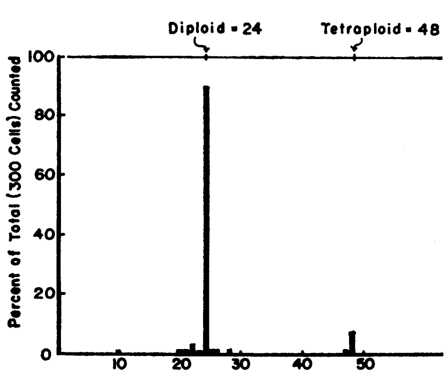

Fig. 2. Distribution of chromosome numbers expressed as percent of the 300 cells counted. Ninety percent of the total have the diploid number 24.

The one cell with ten chromosomes remains somewhat of an anomaly. The obvious explanation would be that the cell wall was broken in the process of squashing and some of the chromosomes were lost from the cell. Since this cell was so unusual, it was examined with special care. No detectable break in the wall was found.

The divisions appeared normal with no bridging or lagging of chromosomes. During the late anaphase and early telophase, one to four chromosomes would frequently spread out ahead of the rest of the migrating chromosomes. This spreading is not peculiar to the tumor cells, but it is also found in microspore cells and in cells from vegetative buds taken from normal-appearing spruce trees.

The mitoses in this strain of spruce tumor cells appear to be completely normal, uniform, and stable, showing none of the irregularities noted in another strain by de Torok and White (*1*). This stability matches the physiological stability of the strain. It also corresponds to the stability noted in strains of crown gall tumors reported by Levine (*6*), Kupila (*7*), and Partanen (*8*), and the diploid number of 24 is the same as that reported by Sax and Sax (*3*) for normal tissues of this genus.

Torrey (*9*) has reported a polyploid drift in cultures of normal pea root tissue grown in a complex nutrient. He attributes this drift to selective influences by the nutrient. Similar drifts have been reported repeatedly in animal tissue cultures (Ford *et al.*, *10*) and they are suspected of playing some role in the emergence of neoplasia. In this respect the instability reported by de Torok and White was not unexpected.

The preparations made by de Torok and White were from primary explants which had been in culture for no more than 1 to 2 months and which had not been subcultured. Their results should represent as nearly as possible the conditions within the tree. The strain considered in this investigation had been under cultivation for more than 40 passages. If a drift toward polyploidy, either innate or under the impact of nutrient influences or selection, had been a factor in the development of this strain, some indication would be expected in this older tissue. There has been none. Either this strain was innately stable or whatever selection there may have been was in favor of the diploids, not in favor of polyploids or aneuploids. This tissue is growing rapidly, uniformly, and apparently in quite normal fashion cytologically. Neither cytological instability nor polyploidy is an essential characteristic of the tumorous state in this tree. Such instability as that observed by de Torok and White was fortuitous, not general.

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References and Notes

1. D. de Torok and P. R. White, *Science* **131**, 730 (1960).
2. J. Reinert and P. R. White, *Physiol. Plantarum* **6**, 177 (1956).
3. K. Sax and H. J. Sax, *J. Arnold Arboretum* **14**, 356 (1933).
4. P. G. Risser and P. R. White, in preparation; P. R. White and P. G. Risser, in preparation.
5. S. J. Kessel, in preparation.
6. M. Levine, *J. Natl. Cancer Inst.* **10**, 1005 (1950).
7. S. Kupila, *Ann. Botan. Soc. Zool. Botan. Fennicae Vanamo* **30**, 1 (1958).
8. C. R. Partanen, in *Developmental Cytology*, D. Rudnick, Ed. (Ronald, New York, 1959), p. 21.
9. J. G. Torrey, in *Cell, Organism and Milieu*, D. Rudnick, Ed. (Ronald, New York, 1959), p. 189.
10. C. E. Ford, J. L. Hamerton, R. H. Mole, *J. Cellular Comp. Physiol.* **52** (Suppl.), 235 (1958).

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Bile Duct Restoration in Rana pipiens after Ligation of the Hepatoduodenal Ligament

Abstract. *A single cotton ligature closed the common bile duct, the hepatic artery, and the hepatic portal vein. The continuity of the bile duct was restored in 14 of 69 cases, the restoration process being associated with mitosis in the bile duct epithelium. There was evidence that the continuity of the blood vessels was also restored.*

The data for this report emerged from a projected experiment on liver homografting. Williams (*1*) described successful grafts made in *Triturus viridescens.* I had proposed transplanting a liver lobe to an ectopic site in the hope that it would acquire vascular adhesions with the host tissues. In order to assess the survival value of such a graft, it would be necessary to determine the average life expectancy in frogs in which the liver had been disconnected from its normal afferent vasculature and the common bile duct.

The hepatoduodenal ligament contains, besides a part of the pancreas, the common bile duct (into which the pancreatic duct empties), the hepatic portal vein, and the hepatic artery. All of these passages can therefore be closed by a single ligature placed around the ligament. Unexpectedly, the animals so treated survived for long periods, one specimen having been killed 231 days after the ligament had been doubly ligated and severed. This failure to establish definite limits to viability raised the presumption that regenerative processes might be at work, and experiments were accordingly continued as a test of this hypothesis.

The general methods of handling and maintaining the frogs, which were adult females, have been previously described (*2*). In the series here recorded the technician used cotton thread for the ligature, which was tied in a square knot, and for closing the incision. Strict asepsis was not attempted. Simple ligation of the ligament without severing was done on all frogs, and the ligature was so placed that bile drainage from the entire liver was blocked (Fig. 1). The ventral abdominal vein was left intact, so that the liver continued to receive blood by way of that vein's connection with the hepatic portal vein above the ligature. The animals were killed at intervals from 1 to 216 days after surgery, and the patency of the common bile duct was tested by squeezing the gall bladder. Transverse serial sections were made of the ligament beginning in the liver insertion and continuing caudally past the area of the ligature as a means of verifying the bile duct continuity where present. A total of 69 frogs was used.

At autopsy the bile could be made to flow normally into the duodenum in 14 cases, and the continuity of the bile duct was confirmed microscopically. The shortest time in which the continuity of the bile duct was reestablished after operation was 10 days (1 of 7 cases), although in this instance some of the bile leaked into the coelom and some went to the duodenum. In the other cases where continuity had been restored, one was encountered at 19 days, three at 31 to 37 days, and nine at 48 to 133 days.

The sections revealed the degree to which the tissues of the hepatoduodenal ligament had grown around the ligature (Fig. 2). In most cases (10 to 133 days) the ligature, or a part of it, could be seen situated within the lumen of the reforming bile duct (Fig. 3). This is interpreted as a stage in which the duct had been in the process of growing around the ligature, an impression which is enhanced by the fact that in such circumstances the bile duct epithelium was incomplete, so that patches of bare connective tissue bordered the lumen. A comparable process of reconstitution of the intestine after ligation has been reported by Goodchild (*3*) in the same species.

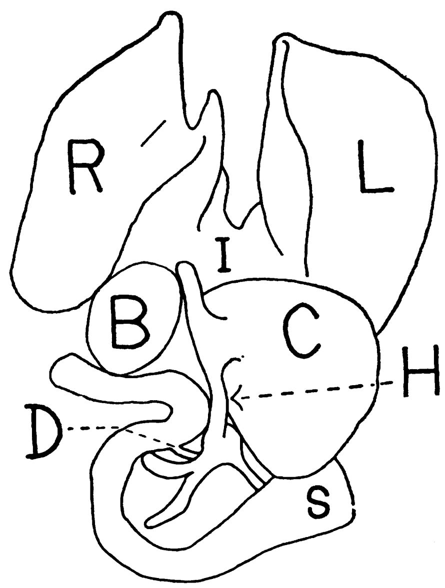

Fig. 1. Diagram of liver dissection, drawn from specimen in ventral aspect. *C*, Center lobe of liver, turned aside to show *H*, the hepatoduodenal ligament. The arrow indicates the position of the ligature. *S*, Lower end of stomach; *D*, duodenal insertion of the common bile duct; *B*, gall bladder; *R*, *I*, and *L*, right lobe, isthmus, and left lobe of liver.

By 5 days after operation the bile duct epithelium showed a high degree of mitotic activity compared with the normal organ. Chiakulas and Millman (*4*) reported a high mitotic index in regenerating gall bladder of larval *T. viridescens*.

However, Panahandeh (*5*) found that ligating and dividing the common bile duct in *Rana fusca* invariably had a fatal issue within a short time (10 to 45 days) after operation.

The 55 cases in which the continuity of the bile duct failed to be restored include 45 animals which were sacrificed in the same time intervals as mentioned above, one at 216 days, and nine at under 10 days. Here the slides showed the terminus of the cephalic stump of the bile duct and, a number of sections beyond, the beginning of the caudal stump. One exceptional case (129 days) showed the cephalic and caudal stumps apparently in the act of passing each other without having met.

The systemic effects of the ligature

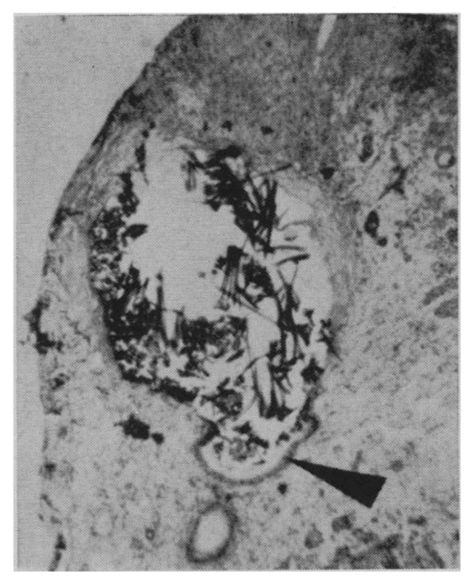

Fig. 2 (left). Cross section of portion of hepatoduodenal ligament; hematoxylin and eosin (× 42), 113-day specimen. Upper pointer indicates part of the ligature. Lower pointer indicates the common bile duct. Fig. 3 (right). Same area as in Fig. 2, 33-day specimen. Pointer indicates the incomplete epithelium of the restoring bile duct whose lumen communicates with a large cavity in connective tissue occupied by fragments of the ligature.

varied in degree and kind according to the postoperative time elapsed, and among individuals of the same time group, so that a single description of autopsy findings would not fit all cases. The gall bladders were frequently empty and colorless, and in some cases could not be identified on gross examination. In other cases they had increased in size, some being otherwise normal, some discolored. The surface of the liver adjacent to the gall bladder was sometimes stained a bright green, suggesting that bile had passed through the gall bladder wall. Microscopic examination revealed a tendency for the gall bladder wall to become thickened and for its epithelium to undergo metaplasia to stratified columnar.

Some of the livers showed varying degrees of damage according to elapsed time and the success or failure of bile duct restoration. A marked increase in the amount of pigment was sometimes present, but evidence from other experiments indicates that this phenomenon is associated with prolonged starvation as well as with obstruction of the bile duct. Discernible jaundice, in the sense of obvious discoloration of body fluids, was encountered in only one instance.

I have recently given attention to interruption of only the bile duct at a point between its emergence from the pancreas and its insertion on the duodenum. The methods include ligation, severing, and removal of a segment of the duct. After such a treatment the continuity of the bile duct is restored sooner and with greater frequency than where ligation is done on the hepatoduodenal ligament.

In some cases an artery and a vein could be traced past the ligature directly into the liver whether the continuity of the bile duct had been restored or not. The presence in the hepatoduodenal ligament of these vessels, if correctly identified, suggests that the afferent vasculature had been restored.

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References and Notes

1. D. D. Williams, *Transplant. Bull.* **27**, 100 (1961).
2. J. C. Streett, *Texas J. Sci.* **10**, 236 (1958).
3. C. G. Goodchild, *J. Exptl. Zool.* **131**, 301 (1956).
4. J. J. Chiakulas and M. Millman, *Anat. Record* **133**, 129 (1959).
5. J. Panahandeh, *J. Pathol. Bacteriol.* **83**, 566 (1962).
6. Supported by a grant from the Brown-Lupton Fund.

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Vision and Touch: An Experimentally Created Conflict between the Two Senses

Abstract. *Observers were presented with an object whose visual shape, because of optical distortion, differed considerably from its tactual shape. After simultaneously grasping and viewing the object, the observers were required to indicate their impression of it by drawing it or by matching another object to it. The results reveal that vision is strongly dominant, often without the observer's being aware of a conflict.*

The experiments in this report are designed to answer the question: If contradictory information is given to two senses of an observer about the properties of an object, what will be his experience? By means of optical distortion, an observer can be given a visual impression of an object which is at odds with his tactual impression of that same object. Will the observer be aware of this contradiction or will one unified impression be experienced? If a unified impression is experienced, will it be a compromise between the visual and tactile sensations or will one sense dominate? Although analogous experiments have been carried out on the problem of localization and on the perceived upright, the conflict between vision and touch concerning properties such as shape or size has not been investigated.

Several experimental procedures were used. In all experiments, the subject viewed a standard object through a transparent plastic optical element which compressed the image along its horizontal axis only, thus changing the object's visual shape. While the subject was looking at the object, he was also instructed to reach behind and to grasp it through a black silk cloth, which prevented the subject from seeing his hand, since any distortion in the visual appearance of his hand could lead to a loss of experimental naivete. The subject viewed the object through an eye piece set into the front of a box. He saw it within the small field provided by a circular opening in front of the optical element. The subject placed his right arm around to the rear of the box which was 40.6 cm deep and, through a large opening (and through the cloth hanging directly behind) grasped the object, a 25 mm white square, 1 mm thick, made of a hard plastic material, attached to a thin black metal stem set vertically in a hole in the bottom of the box. The image of the width was optically reduced by a transparent piece of plastic, 0.6 cm thick, with parallel sides, which served as an optical lens element. The element could be bent around a vertical axis only, by turning a dial to the desired degree of reduction; thus it formed a portion of a thick-walled cylinder. Rays striking the plastic at an angle of incidence other than 90° were displaced. This effectively compressed the image of the object along one axis only. In these experiments the plastic was bent to reduce the width of the image by approximately one half. The plastic was placed 15.2 cm in front of the standard square and 25.4 cm from the observer's eye.

The question of how to measure what the subject experienced was an interesting one. After viewing and grasping the standard, the subject could be asked to select a comparison object which he judged to match the standard. But how should the comparison object be presented, visually or tactually? Eventually we decided on three different experiments: (i) visual comparison only, (ii) tactual comparison only, and (iii) a quite different method in which the subject was asked to draw a picture the same shape as the standard. In this last method, the subject utilized both visual and tactual senses in making his reproduction. Different subjects were used for each experiment. In all experiments, the subject was instructed either to draw or to match in accordance with his "impression" of the standard. In this way no bias was introduced for vision or touch, as would be the case if we asked him to match what he had "seen," or what he had "felt."

In each experiment there were three conditions. In the experimental condition, the subject viewed the standard and at the same time grasped it manually. Pains were taken to insure that he viewed and grasped the standard simultaneously and that he never performed one maneuver without the other. He then selected or drew a rectangle which seemed to correspond in shape to the standard. This was the main condition in which vision and touch yielded conflicting information. The subject was not told what his task was to be afterward until after he had been exposed to the standard. This was to prevent him from using his fingers to measure or otherwise engage in judgmental