Meat Animal By-Products of Pharmaceutical and Food Interest

B. Tie Khouw*, Co-Presenter
Leon J. Rubin, Co-Presenter
Brad Berry, Coordinator

Introduction

Animal tissue and organs are known as rich sources of biochemicals, some of which are of medicinal importance. In this presentation, we shall stress three pharmaceuticals and two enzymes used in the food industry as examples of important packing-house by-products. Their sources and uses will be given with an indication of the methods of preparation. Some other materials which are produced in lower volume are nevertheless of some importance, and they will be briefly mentioned.

From the standpoint of the meat-packing industry, these by-products are low in weight and with variable dollar value, but they can contribute substantially to the overall packing-house economics. This is particularly true when proper care is taken in handling, collecting, preserving and storing organs and tissues which are the raw materials for further processing. The biochemicals contained within them are very labile. They are susceptible to the action and proteolytic enzymes and to microbial degradation often associated with the improper handling of these organs and tissues prior to chilling or freezing. It is therefore important to maintain the content of the labile biochemicals within these by-products if one hopes to derive from them their full monetary value.

The by-product biochemicals that we shall focus on today are by no means new, but they are important in the management of human disease and in the production of food products. Each enjoys a substantial annual market in excess of US 50 to 100 million dollars. Please note that all monetary values will be given in US dollars. Although the currently emerging biotechnology industry may offer an alternative source of these materials, this is still some years away.

The products we will concentrate on today are the pharmaceuticals – insulin, kallikrein and heparin; and two enzymes – chymosin and catalase used in the dairy industry. They have been selected here because we have been closely associated with their preparation and commercialization. There are many more important and interesting biochemicals which can be derived from various animal by-products, as we have pointed out earlier (Rubin, 1977).

Insulin

Insulin is a protein hormone concerned with the regulation of the rate of carbohydrate metabolism. The lives of diabetics depend on insulin. The protein contains 51 amino acids arranged in two chains, A and B, with 21 and 30 amino acids respectively (Fig. 1). The hormone is produced in the pancreas as the inactive form known as proinsulin. This is converted into the active insulin and then secreted into general circulation in the body. It is estimated that we produce between 1-3 mg of insulin/day. This quantity is probably of the same order of magnitude that an animal such as a cow or a pig produces. In the case of a cow of 500 kg body weight, the weight of the pancreas is about 0.2 kg or 0.04% of the body weight. The insulin content is about 20 mg, or 0.01% of the weight of the pancreas. A small number indeed! The insulin requirement of a diabetic patient is about 3 mg/day. There are approximately 2 million users in the United States and about 15,000 new users are added each year. These numbers represent about a $480 million annual market (Lunzer, 1988).

Since its discovery in 1921 by Banting and Best in Toronto, the insulin production method has been developed and refined but, by and large, it has not changed drastically. Briefly, pancreas (hog or beef) is collected, trimmed, and quickly chilled and frozen. The frozen gland is chopped and ground, and extracted with acid alcohol. The extract is clarified, and the alcohol is removed under vacuum at room temperature to preserve the insulin in the aqueous extract. It is then precipitated by the addition of salt as a cake, and the crude cake is subjected to extensive purification to obtain insulin protein with a purity in excess of 99.9%. It is important to bring the contaminating proteins down to very low levels because these minute contaminants can induce immunologic reactions in some patients. Indeed, it is because of this very concern that better insulin preparations have been sought. Two approaches are taken. Human insulin differs from porcine and bovine insulin by 1 and 3 amino acids respectively (Fig. 1). The porcine insulin has been successfully converted to human insulin by exchanging the last alanine in the B chain by threonine, which is the amino acid present in the human insulin. In a different approach, the molecular biologists have engineered two genes coding for the A and the B

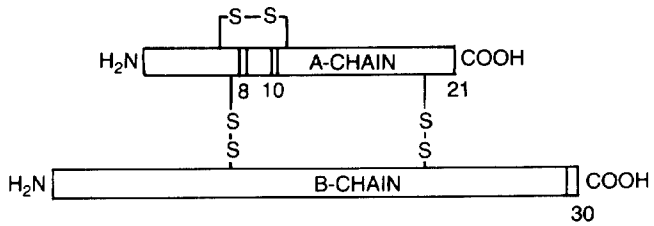
*B.T. Khouw, Waitaki International Biosciences, 55 Glen Scarlett Road, Toronto, Ontario, Canada M6N 1P5

L.J. Rubin, Department of Chemical Engineering, University of Toronto, Toronto, Ontario, Canada M5S 1A4

B. Berry, ARS, USDA, Meat Science Research Lab. Bldg., 201 ARC-E Beltsville, MD 20705

Reciprocal Meat Conference Proceedings, Volume 42, 1989.

Figure 1



A-CHAIN:	Human	Porcine	Bovine
No. 8	Thr	Thr	Ala
No. 10	Ile	Ile	Val
B-CHAIN:			
No. 30	Thr	Ala	Ala

Structure of insulin molecule showing the arrangement of the A and the B chain consisting of 21 and 30 amino acids respectively. Differences in amino acid contents within these two chains for insulin derived from human, porcine and bovine origin are also listed.

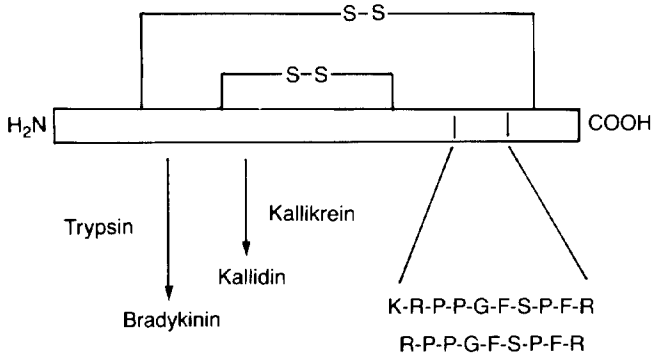
chain of human insulin. On introduction to two separate bacteria of the same species, these two chains are produced from which human insulin can be made. This latter form of humanized insulin is currently available, but the production cost is much higher than for the insulin produced by the extraction technology. It is, however, the trend now for physicians to prescribe for all new diabetics the humanized insulin instead of the naturally-derived product.

Kallikrein

Besides insulin, the pancreas also produces, stores and secretes many more enzymes and hormones. One of these enzymes is called kallikrein, a Greek term for the pancreas. This proteolytic enzyme was discovered in the early twenties in Germany by Professor Frey. While working on the association between the heart action and the function of kidney, Professor Frey found that on injecting a dog with urine from another dog, there was a decrease in blood pressure. Subsequently, a similar blood-pressure reducing substance was also shown to be present in the pancreas, as well as other tissues and organs. This was initially given the name F-substance for Frey, and later renamed kallikrein in 1930 (Werle, 1970).

Kallikrein is a carbohydrate-containing protein of about 30,000 molecular weight. It is a very specific proteolytic enzyme. The only known substrate, present in plasma, is called kininogen. Thus the enzyme is also known as kininogenase. The enzyme hydrolyzes this substrate to form a ten-amino acid peptide known as kallidin. A nine-amino acid peptide, bradykinin, is generated by another well-known pancreatic enzyme, trypsin (Fig. 2). These kinins are the vasoactive substances, capable of dilating smooth-muscle tissues, which lead to a reduction in blood pressure. If it is generated in large amounts, μg instead of ng levels, the kinins are associated with pain. Because of the hypotensive action, the enzyme has been used as an antihypertensive agent in the 1940's in both Europe and Japan. Based on the amount sold annually, it can be estimated that the current Japanese market for kallikrein is in excess of 100 million dollars annually. Indeed, there are some 10 different companies in Japan involved in producing and/or marketing kallikrein under various names, a big business indeed. The enzyme is marketed as a drug in the form of tablets, and has been under review by the Japanese health authority (the equivalent of FDA) for efficacy. Thus far, no definite ruling has been forthcoming.

Figure 2



Structure of a low molecular weight kininogen substrate showing the location of the kinin fragment.

Kallikrein, like insulin, is present in the pancreas in small quantities, about 100 mg/kg tissue. It can be isolated by a variety of methods. In a method which we have developed, frozen hog pancreas is chopped and extracted with aqueous buffer. The extract is then fractionated with ammonium sulfate and a crude kallikrein fraction is obtained. This is purified by an ion-exchange resin, and subsequently recovered as a freeze-dried powder (Khouw & Kesler, 1974). In Japan, the enzyme powder is formulated with other ingredients in tablet form containing the equivalent of 10-50 μg of pure kallikrein/tablet. These tablets are taken orally 1 to 2 times daily. When faithfully taken, the preparation is claimed to help in maintaining health and well-being. This claim is not universally accepted.

Heparin

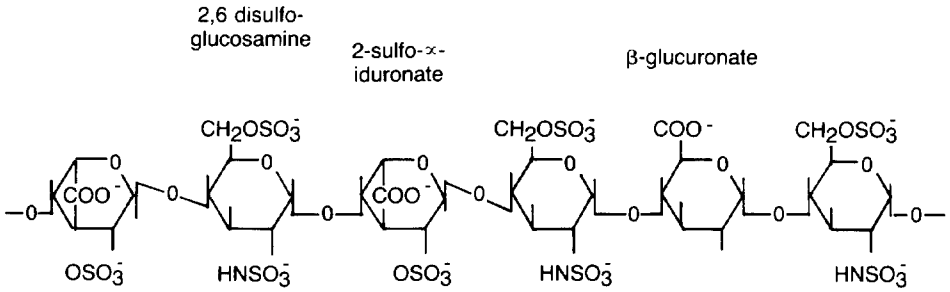
Unlike insulin and kallikrein, heparin is a non-protein pharmaceutical. It is a very acidic, highly sulfated anionic polysaccharide, also known as glycosaminoglycan. In fact it is the highest negatively charged molecule in tissues. Briefly, as isolated, heparin consists of repeating sugar units containing hexosamine and hexuronic acids. The smallest functional unit is a molecule with 6 sugars as shown in Figure 3. Heparin is a heterogeneous preparation, ranging in molecular weights from about 3000 to 35,000 with an average of about 12 to 20,000 (Jacques, 1979).

Heparin has the property of preventing blood clotting. It is a potent anticoagulant. It is this very property that resulted in its early universal clinical use. In the normal process of blood clotting (a complex process), the final step involved is the conversion of fibrinogen to an insoluble fibrin clot by the action of the proteolytic enzyme thrombin present in the plasma. Heparin, when present together with antithrombin, inhibits the action of thrombin on fibrinogen. To produce anticoagulant activity, heparin is used at a level of 10 to 20 μg per ml of plasma, given by injection or infusion.

Heparin is widely distributed in mammalian tissues and

Figure 3

STRUCTURE OF HEPARIN HEXASACCHARIDE



Ref. – Jaques, L.B., 1979. Science 206:528-533

fluids, the largest amounts being in lung, spleen, liver, muscle and intestine. Beef lung was used earlier as a source of heparin, but the purification of heparin from such a source is difficult and the process is no longer considered economical. It is now customary to isolate heparin from hog intestinal mucosa. The mucosa is low in solids and carries a heavy bacterial load. It needs to be well preserved. This is normally carried out by the addition of 1% sodium metabisulfite.

The isolation process generally involves an initial alkaline hydrolysis of the mucosa to liberate heparin from the protein complex, and the free protein is then digested by proteolytic enzymes to facilitate heparin recovery. The method of choice now is to use an ion-exchange resin to adsorb the heparin in the dilute extract, and subsequently to recover it by elution with a concentrated salt solution. The eluted heparin is precipitated by alcohol to obtain a crude heparin. This is then extensively purified in a series of steps, one of which is a bleaching step with either permanganate or peroxide, to give a pharmaceutical grade of the sodium or calcium salt of heparin. The final product is a white powder with an activity of not less than 140 USP units/mg.

The present world-wide market is in excess of $100 million per year. In the United States alone, more than 900 billion units (6 metric tons) are used annually (Jacques, 1979). The demand is steady, and the supply is plentiful. After all, there are over 300 million pigs in China, and China is certainly one of the big players in the world market.

A new form of heparin has been developed and is now in use in Europe. It is the low-molecular-weight form of about 5 to 10,000 instead of the 12 to 20,000 daltons. It is obtained not by genetic engineering, but by the old-fashioned chemical method of the nitrous acid depolymerization reaction, or by an enzymatic digestion with heparinase. This new form of heparin is more efficacious and specific in its action as an anticoagulant. It produces no unspecific bleeding and other complications often noted in some users when the normal heparin is used.

Chymosin and Catalase

Turning now to the non-pharmaceutical products, we will deal with two enzymes used in the dairy industry. These are chymosin used to clot milk for cheesemaking, and catalase used to decompose hydrogen peroxide which is used to "sterilize" the milk prior to its coagulation by chymosin.

Chymosin (or rennet, rennin) is a proteolytic enzyme present in the 4th stomach (abomasum) of calves. This is the primary enzyme in the calf rennet extract responsible for the clotting of milk, especially when a high-quality cheese is desired. The other minor enzyme is pepsin, which also is capable of clotting milk and is widely used in cheesemaking. Like kallikrein, chymosin is a specific proteolytic enzyme capable of hydrolyzing a specific peptide bond in one of the casein proteins present in the milk. The actions of chymosin in cheesemaking involves the single bond splitting of kappa-casein to para kappa-casein and a macropeptide. In the presence of calcium, the para kappa-casein is converted to a dicalcium-para-kappa-casein which is insoluble, forming a clot or curd. Many other proteolytic enzymes, including pepsin, are capable of accomplishing this conversion, but they are not specific enough and their hydrolytic activity continues making the curd or clot soft, and often generating bitter peptides (Godfrey and Reichelt, 1983).

The enzyme is traditionally obtained by extracting the chopped 4th stomach with a salt solution. The enzyme, obtained in an inactive state in the extract, is then activated by acid and separated from its main contaminant, a mucin-like substance. This is carried out by precipitating the enzyme at a high salt concentration, and removing the mucin with potassium alum. The purified enzyme can then be crystallized, if desired (Berridge, 1985). In a more recent method (Clarke, 1976), the stomach extract is treated with an anion-exchange resin to adsorb chymosin but not the mucin. The adsorbed chymosin is then eluted with a salt solution and the enzyme is precipitated by the addition of more salt. In this way, a relatively pure chymosin is obtained without involving many of the tedious and time-consuming steps normally associated with the traditional method.

The demand for chymosin is strong, especially when good-quality cheese is required. The supply of chymosin is limited by the availability of milk-fed calves. There are now intensive efforts under way by several companies to use genetic engineering to produce chymosin using bacteria in a way similar to the production of human insulin already referred to above. There are also intensive efforts being made to modify the various microbial rennet-like enzymes so that they would behave more like chymosin.

We shall now turn to the other enzyme, catalase, which is also used in the dairy industry. Catalase is a large protein of about 250,000 molecular weight. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. The enzyme is present in all aerobic organisms, but it is present in large

concentrations in the liver of mammals. Beef liver is the best commercial animal source. The enzyme is composed of 4 identical subunits, each containing a heme protoporphyrin group, similar to hemoglobin. When isolated in a relatively pure form, the enzyme is greenish instead of reddish as is hemoglobin derived from the blood. The enzyme is considered to be the most active and fast-acting enzyme. It was first crystallized by Sumner and Dounce (1937).

The enzyme is conveniently isolated from beef liver by a process similar to the one used by Sumner and Dounce. It involves an aqueous extraction of minced liver, followed by solvent (usually acetone) fractionation. The enzyme is readily crystallized from dilute salt solutions. The isolated catalase, purified or crystallized, is stabilized and standardized in a buffered solution. It is marketed in North America by Miles under the name Catalase L.

The use of these two enzymes in the dairy industry is well known. Briefly, 5% hydrogen peroxide is added to milk at a level of 0.4%. This is kept at 30°C for about 20 minutes to let the peroxide "sterilize" the milk by killing the harmful bacteria but leaving the advantageous microorganisms unharmed. At the end of 20 minutes, a catalase solution of a specific strength is added to decompose the residual peroxide. The treated milk is then ready for curdling by the action of chymosin.

Other By-Products-Derived Pharmaceuticals

There are many more by-product pharmaceuticals currently in use in the various parts of the world. They enjoy a good steady market. We can only mention them here in the form of a partial list (Table 1). Many of the big pharmaceutical companies market these products under their various brand names.

The Impact on the Meat Industry

Having examined the various biochemicals one can obtain from the by-products of the meat industry, let us now turn our attention to the impact on the meat industry itself. What benefit can they bring in terms of revenue?

Since these by-products are low in weight, usually less than 1% of the animal weight, their monetary contribution per animal will be small. As an example, the weight of pancreas in a hog is about 0.1 kg and in a beef animal, 0.2 kg. Taking the present market price of hog and beef pancreas at about $4 and $1.50/kg respectively, the saving of pancreas leads to a return of about 40 and 30 cents per carcass for pigs and cows respectively. This may not be much, but when we consider the large number of animals that are available, the total return can be substantial. Furthermore, by-products collected on this continent are considered disease-free, and some of them can often command a premium price when properly presented in the export market.

Table 1. Other By-Product-Derived Pharmaceuticals.

Source	*Product*	*Uses*
Blood	Thrombin	Blood clotting enzyme
	Albumin	Blood group (Rh) typing
	Fibrinolysin	Blood clot dissolution
Pancreas	Deoxyribonuclease	Debriding enzyme in combination with fibrinolysin
	Glucagon	Insulin antagonist
Lung	Aprotinin	Pancreatitis treatment
Testes	Hyaluronidase	Spreading factor to aid drug dispersion in tissue
Bile	Cholic acid	Intermediate for cheno- and ursodeoxycholic acid
	Chenodeoxycholic acid	Gallstone dissolution

Other aspects to consider are the potentially high cost of disposing of high BOD-containing waste, such as blood, when not collected for food use, or the high energy required in rendering a low-solid-content by-product such as intestinal mucosa when not used for heparin production. Thus the saving of these by-products and their utilization can often result in a positive return. Finally we must not forget the value that some of these by-products bring in terms of human welfare.

Conclusions

In this discussion, we have concentrated on five biochemicals which have a substantial market, in the order of 50 to 100 million dollars world-wide. There are many more items one can obtain from animal by-products. The blood is a rich source of numerous proteins which have their own market segments, and some of these are listed in Table 1. There are numerous diagnostic enzymes used daily in clinical chemistry. Many of these are derived from various animal by-products. We are confident that animal by-products will remain a source of viable commercial biochemicals for many years to come, and will be a source of additional revenue to the meat industry.

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Discussion

D. Shaw: If the pharmaceutical industry is still dependent on the animal slaughter industry for these byproducts, is the slaughter industry supplying enough? I know that there are a lot of packers that have quit saving pituitary glands and pancreas. Is the supply sufficient or is there still a need?

B. Khouw: The supply of some items is still plentiful. We can take a look at heparin, which is derived from hog intestinal mucosa. It has been estimated that in the United States the amount of heparin used per year is roughly 6 metric tons. To derive this amount of material, there is no problem because plenty of hogs are killed. In fact, there are over 300 million pigs in China alone and the Chinese are a big player in the world heparin market. Presently, saving of some organs is not in fashion, partly because the chemicals that can be derived from these organs do not occupy a sizeable amount of market, so the demand is not that great. As a result, in packing houses they do not save organs because the return is not justified. But in the case of pancreas, it is still being collected for insulin and for many other enzymes; the lung is still being collected and blood, while still underutilized, with the development of the biotechnology is becoming a source of proteins to use as a growth component in mammalian cell culture. Many of the growth factors present in bovine or porcine blood can now be derived. So the requirement for chemicals has shifted; before, we tended to concentrate more on pharmaceutical items, now we try to concentrate more on the biotechnical side.

D. Buege: What proportion of the livestock that are slaughtered really avail themselves of recovering these materials? What is the outlook for the future value of them in light of the fact that genetic engineering might be producing an alternative or perhaps a superior product?

Khouw: In terms of the genetically-engineered compounds, I think there is still a long way to go; partly because the people who are in this field are trying to concentrate on the human pharmaceuticals, and commonly the use of animal-derived products in pharmaceuticals is rather limited (such as heparin and insulin). The production costs at present for genetically-engineered insulin are still very high compared to the extracted insulin and the benefits for this insulin appear to be minimal. In other words, the so-called humanized insulin does not behave any better than the traditional insulin and, of course, the costs are much higher. The supply of extracted insulin is plentiful because there is a plentiful supply of pancreas both from the porcine and bovine species that can be used to produce the insulin. It appears that the number of diabetic patients are confined to the Western world and very little is observed in the developing countries or in Asia. Eventually, I think that many of the pharmaceuticals will be replaced by the genetically-engineered compounds, but I think this is still some time off in the future.

L. Rubin: I'd like to add a comment regarding the collection, especially of pancreas. A very thorough job is done of this. Eli Lilly has two people on the road all the time visiting packing houses, large and small, teaching them how to trim, collect, freeze and preserve the glands. So as far as pancreas is concerned, a very thorough job is done.

Khouw: On that point, I would like to add that it is important to teach people how to collect the organs and tissues properly, because to people like us who process the material, there is very little we can do. If we take a 3000-pound batch of pancreas for extraction (the pancreas comes in 50 lb. boxes), it is impossible for us to really go through every box trying to determine the quality. Suppose we extracted 3000 lbs. of pancreas and found out they contain only half the amount of insulin; certainly, we are not going to dump the whole thing down the drain. But we have to go through the entire process, that's why the collection becomes very important and that is the reason why the Eli Lilly people have two full-time people going around to ensure such a proper collection.

N. Firth: To what extent are these byproducts being done away with by competition from synthetic substitutes?

Khouw: Right now it is still very little because people who are working on so-called synthetic by-products, by genetic engineering or by chemical engineering, are not looking at the products that we are dealing with here, except in the case of insulin. However, the cost of producing insulin by genetic engineering is still very high; at least twice as high as insulin obtained by traditional extraction technology. Also, in the case of heparin, we have to rely on the natural source because up to now we do not know how heparin is made in the body, let alone try to engineer it genetically. Unless somebody of course comes up with a better natural anticoagulant.

Interestingly enough, heparin has the property of preventing blood clotting. But that is not the physiological function of heparin. We know about the chemistry of heparin but we do know the function of heparin in the body. In the body, we have a natural coagulant that is in the form of protein; it is called protein C and that protein is now being genetically engineered as the natural coagulant rather than heparin, but there is still a long way to go.

Rubin: How close are we to seeing chymosin as a genetically-engineered product?

Khouw: I think within the next few years because right now they are doing the safety aspect of it. Once that work is complete, they will get FDA's blessing to market the product.

D. Kropf: I assume that when you get these organs, raw materials, they need to be kept cold and probably frozen. Are any of them vulnerable to freezing?

Khouw: The best system is upon collection to treat the organs properly; remove all the extraneous fat and chill it as soon as possible. If kept frozen, most biochemicals are not destroyed by freezing. In fact, we can keep the organ frozen for more than 1 year without affecting it.

Kropf: That was my second question. How long can we keep this material? Are any of these materials more vulnerable to length of frozen storage?

Khouw: I guess it depends on the type of material we have, but in general they are not sensitive to frozen conditions. There are certain items such as ferritin, which is an iron-containing compound derived from the spleen, and if we keep this frozen for too long, we cannot isolate the ferritin in a good crystalline manner. So there are some items that are

sensitive to freezing. But by and large, most of them are not sensitive to freezing.

Buege: As a follow-up to that, is there a time limit as to how long you can keep pancreas in frozen form before quality deterioration occurs?

Khouw: As long as it is kept frozen all the time without thawing, pancreas can be kept in frozen storage.

Of course, there is a difference in insulin and enzyme content due to animal age. Younger animals tend to have a higher content of insulin but lower content of enzymes. But pancreas derived from the older animals have a higher content of enzymes but a lower content of insulin. Usually when we want to make insulin, we collect pancreas from younger animals and those who want to make enzymes would collect pancreas from the older animals.

B. Berry: You mentioned the need for rapid freezing. Are there any other unique processing steps that should be followed or specialized facilities for some of these pharmaceuticals that might prohibit some packers from collecting these materials?

Khouw: I think the simplest procedures are to chill the materials as soon as possible and then freeze them immediately. We can bring in all kinds of preservatives that will preserve the various biochemicals but many of these compounds are considered to be toxic and not acceptable for food use. So by far the best method still is the physical method of chilling it as fast as possible.

Rubin: You should perhaps expand on this a little bit as far as hog casing slime for heparin. It is a totally different material; it is very dilute; it only has about 10% solids in it. One has to collect and transport a lot of liquid which is very perishable. So you have to add a preservative which is a 1% sulphur dioxide material (sodium metabisulphite). That is the usual way of transporting it. That's one of the limitations of collecting this material, you can only collect it within a certain radius of the processing plant.

Khouw: We have calculated that every cent-per-pound increase in the cost of this transportation translates approximately to $1 increase in cost for heparin.

Rubin: I'd like to ask a question of my partner here since he has been in the business for 10 years longer than I have. I understand the cost of porcine pancreas has increased remarkably. Is it because of its use as a source of insulin for making humanized insulin?

Khouw: Yes, porcine pancreas right now is selling for about $4 per kg (US$). Last year it was as high as $12 per kg. We have not been able to determine exactly why the cost of pancreas suddenly went up sky high but there are several theories. Eli Lilly decided to stock up their supply of porcine insulin so they built up their inventory and I think this is perhaps one of the reasons why the cost of pancreas escalated. Secondly, I think that the Japanese are interested in obtaining pancreas from this part of the world and with the appreciation of the yen, it certainly becomes very attractive for them to stockpile pancreas. But right now, the porcine pancreas cost has come down to about $4 or so per kg. This is much higher than the cost of beef pancreas which is about $1.50 per kg. What Dr. Rubin said is possible; that some people might want to use porcine pancreas to derive porcine insulin so that they can convert it into humanized insulin. In particular, people in Denmark have such a technology to do the conversion of porcine insulin to human insulin.

Kropf: What methods are used to standardize the activity of insulin? I guess that raw materials can differ widely in their activity and obviously this must be controlled to give to patients. What sort of methods are used to measure activity and how frequently is this done?

Khouw: During the processing stages, a radioimmunoassay is used to monitor the insulin chemically; but once it is obtained in the pharmaceutical grade in crystalline form, it is then standardized by biological activity system — this is the USPSA which is based on the mice convulsion test. I think they use a population of mice which are subjected to insulin shock and they want to make sure that half the population die. This is the biological test. Sometimes activity is measured on rabbits by following the disappearance of glucose molecules in the blood. But during normal preparation, usually we use radioimmunoassay.

Buege: Would you have any information on past or present use of anterior pituitary glands from meat animals and what might be obtained and utilized from these glands?

Khouw: There has been recent interest because in these glands a subgrowth factor has been isolated that is required by some mammalian cells. Other companies are involved in the clinical side to make use of these growth factors. They use it for wound healing but it is still under clinical testing. Several so-called growth factors are being derived from brain and lung tissue. Certainly the type of chemicals that can be derived from byproducts has changed. In the old days, it was the traditional pharmaceuticals and the food-related chemicals; but now with the emergence of biotechnology, a new set of chemicals are being acquired. Definitely, many of these chemicals are still derivable from various byproducts. I think one of the more interesting ones, perhaps, is the insulin-like growth factor. That molecule system is present in beef blood and one can derive and isolate it from beef blood for use in the animal health or animal nutrition field.

Rubin: Would you care to say a few words about the blood from unborn calves?

Khouw: One of the high-selling items is fetal calf serum. Our company in New Zealand produces a sizeable quantity because we have a large population of fetal calves in that country. It is basically used for growing mammalian cells in culture. They have to be collected under sterile conditions through heart puncture so that we don't inherit the bacterial, virus or microplasma contamination into the blood. The cost of fetal calf serum is also very high. I think right now it is selling for about $200 a liter; whereas, the ordinary adult serum is probably selling for about $1 to $30 per liter. Many companies, including ourselves, are working very hard trying to modify the adult serum into a fetal calf-like serum, not necessarily the same in composition, but at least in functional properties.

Rubin: How big a market is fetal calf serum?

Khouw: It is very big. I could not say offhand, but certainly over $100 million a year.

K. Pierce: I had worked at a plant where they were collecting fetal blood. We were doing about 80 boning cattle a day. It was a dairy area so we were probably getting anywhere from 2 to 8 calves per day. The investment in the equipment, which is all regulated and you have to use stainless steel with a vacuum pump, more than paid for itself

in less than a month. From a 120 lb. calf you could get probably 3 to 4 liters of blood. When you said it is $200, were you talking about per liter? Is that the refined product or is that the price you are paying now for the raw?

Khouw: No, that is for the refined product.

Pierce: At that time, and this is several years ago, we were looking at $25 to $40 per liter for the raw product.

J. Carpenter: You did not get into it, but what about the use of blood proteins as a by-product?

Khouw: In blood, currently, there are three items that are being used as pharmaceuticals: thrombin, albumin and the phybrinolycin. I think there are many more biochemicals one can derive but their volumes are on the lower side. For these additional products, their market is less than $50 million per year. These different items are marketed by various pharmaceutical companies in the world under their own brand name. The market for albumin as a diagnostic chemical and as a vaccine component to produce vaccines is probably about 10 to 12,000 kg per year. Several other blood protein components are marketed to various biochemical supply houses and the biotechnology industry for growth factor components to grow mammalian cells. Some of the protein that they are trying to engineer genetically has to be grown in mammalian cells.

Rubin: Seeing that John is very interested in blood fractions, perhaps you should say something about the gammaglobulin from beef as a treatment for calf scours.

Khouw: We also have isolated the gammaglobulin fraction and when this is reconstituted with insulin in saline solution, we can inject it into a calf to prevent calf scours. Blood protein is currently being under-utilized. But only now are we beginning to make use of the blood proteins. It is very difficult to obtain clean plasma even if we chill plasma carefully. Usually by the time we get the shipment of plasma in a 500-liter tank, the bacteria load can be very high (20 million per ml). Presently, we cannot add any preservatives into the blood to prevent bacteria from growing. We can only rely on refrigeration.

Firth: Are pork blood and beef blood equally good as a source of byproduct?

Khouw: I would say, yes, although we have very limited experience working with porcine blood for the simple reason that we do not collect porcine blood as such since it is not being used for food items. To collect porcine blood, we have to get special permission from Agricultural Canada. In the normal process of collecting blood from animals, we have to segregate blood derived from each animal separately until each individual animal has passed inspection. We are now individually collecting beef blood because of the larger quantities. Approximately 10% of the body weight is blood and this is used, more or less, as the general rule of thumb. There have been several interesting biochemicals derived from the porcine blood; one of them is known as the platelet-derived growth factor. This appears to be involved in the wound healing process; but the chemical is also known to be associated with cancer of some sort, so I am not sure where we stand on its future use. All the traditional products which have been derived from beef blood can be derived from porcine blood. Beef blood has been used, not because it is better, but because it was first to be used.

Rubin: I might add to this that in the case of collecting beef blood, the blood from each animal is kept separate until the animal passes inspection. In the case of a hog blood, I think they are grouped in tens, or some similar number, and if any one of those animals does not pass inspection, the whole lot is thrown out. It's also my understanding that in Europe hog blood is collected for food use but not on this continent. We are too rich.

A. Gordon: You have been stressing the pharmaceutical and research uses of animal byproducts. Could you say something about the food uses of these byproducts that you have been discussing?

Khouw: Yes, perhaps the best one is catalase which I didn't have a chance to get into. This is used in conjunction with chymosin. Hydrogen peroxide is used in cold sterilization of milk. Before it clots with the chymosin, we have to destroy the hydrogen peroxide that we used in this milk sterilization. We use the enzyme catalase derived from the bovine liver. We can obtain catalase from a microbial source, but, generally the animal-derived catalase is a better preparation. This is obtained usually by means of a solvent extraction and fractionating and finally we crystallize it and later dissolve it in a stabilizing solution to use. In North America, it is marketed by Miles Laboratory under the name of Catalyst L. We supply such a preparation to Miles Co., which they then reconstitute or make it up to the strength that they want for use in the dairy industry. They also use hydrogen peroxide in England for the treatment of whey, and again they use the catalase to destroy the peroxide. The food use of enzyme for hydrolysis has been replaced by bacterial-type enzymes because they are less expensive to produce.

D. Kinsman: In your last notation on the overhead, we find the gallstone dissolution. I presume this is in human use but it does raise the question, are gallstones still a valuable byproduct to the industry for export to the Orient?

Khouw: Yes, the latest numbers that I could get are something like $3000 a kg for beef gallstones. They are shipped to Japan, China, Asia.

Rubin: They are used as an aphrodisiac?

Kinsman: That's why I'm interested.

Rubin: This can be a serious problem for instance in the case of the rhinocerus. The horn is supposed to be an aphrodisiac (the round horn) which leads to terrible consequences. The animal is being exterminated as a species. They can fetch $3000/kg for the horn. I would like to go back to a question that was asked here by Firth, whether synthetics are replacing the extracted material we are talking about here. To some extent, this is certainly true. Nobody makes sex hormones any more from testes or other sex organs. It is all made now from diosgenin which is isolated from a root and it is a much better raw material. On the other hand, testes are available as a source of hyaluronidase, which is a complex material. I believe it is a glycoprotein.

Khouw: It is a glycoprotein of about 60,000 molecular weight.

Rubin: Use of this material is not in the near future by any means. In the case of insulin, it will be quite a while before insulin derived from the pancreas is replaced by bioengineered insulin. To make it, you have to make the two chains independently and then combine them chemically and I think the cost of these steps and the cost of purification is very high.

Khouw: For the simple molecules, the synthetic ones have replaced the extracted material. A good case in point, of course, is the chenodeoxycholic acid. One can derive chenodeoxycholic acid from hog bile, but it is now more common to produce it synthetically from cholic acid. In fact, one can probably make it synthetically at half the price of the isolated materials.

Rubin: There was a tremendous amount of interest in chenodeoxycholic acid for gallstone dissolution. In the 70's, even in Canada, there were about 65,000 gallstone operations per year. That's a lot of operations and the idea was that it could be replaced by treatment with chenodeoxycholic acid. But, in fact, it did not run out to be quite so simple; but perhaps Dr. Khouw would like to comment on this.

Khouw: When the original studies were carried out, the proper dosage was not determined to achieve the effect that we wanted. It was a bit on the low side. Secondly, gallstone diagnosis is usually not a part of one's annual physical checkup. So for the treatment to be effective, one has to take chenodeoxycholic acid at about 200 mg or 250 mg per day in the form of tablets for several months before the gallstones would be dissolved. We only notice a problem when we get a gallstone attack from the pain; by the time we reach that stage, we are not going to wait several months to take the medication and suffer the pain so we resort to surgery. Because of this, the gallstone as a therapeutic item has never really taken off the ground. But such a preparation in the form of urcel deoxycholic acid is available in the United States. In Japan certainly, and in Europe, the post-preparation cheno and urcel forms are being marketed and have already been on the market for almost 10 years. And this dissolution is true only if the gallstone is made up of cholesterol; which is the case in about 90% of gallstones.

G. Trout: I was wondering whether there is any work being done on bioconversions with some of these compounds like cholic acids. I know work was done on using microbes to convert them. Has that ever been commercialized?

Khouw: It has been worked out — several people have worked on this, but somehow the process has never been scaled up and used commercially. Right now, I think the chenodeoxycholic acid conversion is done chemically.

Rubin: I might add that bile acids were a substantial business and rather an exciting group of chemicals at one time. For instance, beef bile acids from which we get cholic acid and deoxycholic acid were the primary source of corticoid hormones. That's from the deoxycholic acid that the Merck group first synthesized. Cortisone and cholic acid of course can be converted to the deoxycholic acid. But, again, this has been replaced almost entirely now by other materials from plants which are more readily converted to cortical-like materials.

B. Marsh: I have a question for Dr. Reuben. Leon, when I worked with you about 25 years ago when you were Director of Research, Canada Packers, I have quite a strong recollection that you told me about a preparation of enormous scale you were making of cytochrome C for export, I think, to Japan where somebody had the idea that it would assist heart attack victims. Do you have any recollection of making a large supply of cytochrome C and if so, did the market ever develop for that?

Rubin: Well, Bruce, I have been away from Canada Packers for 10 years and Dr. Khouw is much closer to it. We would get these requests and Japan was always a lively source of them. They would come up and die down again. As far as I can recall, cytochrome C did not develop into a big market, but I would like to hear Dr. Khouw's comment.

Khouw: No, it is still now being sold in Japan as an antiinflammatory agent, but the market has more or less toned down a little bit. So as a result, many of the international players who used to supply the Japanese market with cytochrome C have pulled out and because of that we did not get involved in the production. It was processed from beef heart muscle.

Rubin: Kallikrein is produced in sizeable amounts for large markets such as Japan. It is used there but is still under scrutiny by the equivalent of the FDA and in 10 years they have yet to pass judgment.

Berry: One last comment. I happened to have the opportunity to participate on a committee that was involved in putting together this report for NCA. One of the things that was discussed was the term "byproducts." It is one that always had a little bit of a negative aspect to it. The concept was expressed of trying to explore using a term like "allied" products — something in the way of a nomenclature change. I am speaking here primarily for food use. That might be something all of you might want to think about.